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Proceedings of the Eighth Annual Aquatic Toxicity Workshop: November 2-4, 1981 Guelph, Ontario

Compte rendu des communications du huitième atelier annuel sur la toxicité aquatique: du 2 au 4 novembre 1981 Guelph (Ontario)

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The use of Peter Clark Hall was donated by the University of Guelph, and the Organizing Committee extend their grateful thanks to the organizations for their sponsorship.

PREFACE

This is the eighth in a series of the proceedings of the Annual Aquatic Toxicity Workshop. The Workshop provides a forum for informal discussions on subjects relevant to Aquatic Toxicity with particular reference to the Canadian and North American environments. In addition to these discussions, the Workshop also provides a forum for the presentation of the results of scientific research and study.

No restrictions were placed on the papers submitted to the Workshops, however, the submissions were grouped into the major areas of: the effects of pesticides and other pollutants in the aquatic system, single organism bioassay techniques, toxicokinetics, acid precipitation and environmental interactions of pollutants.

The topics for the informal workshops were suggested by registrants and, with the exception of the student-oriented career workshop, are summarized in this volume.

Attendance at the Workshop was 148 registrants, the majority of which were from Ontario. In addition to these it is estimated that 20-30 students attended the Workshop on a casual basis. Papers presented totalled 36, posters 7, and 6 workshop discussions were held during the period of the Workshop.

EDITOR'S COMMENTS

The contents of this volume consist of four main sections.

The first section contains the submitted papers and, in accordance with the policy of this Editorial Committee, have been subjected to external review by one or more referees. While close attention has been paid to the scientific aspects of these papers, few editorial changes of style, etc. have been made except with regard to units and typestyle. Although the review process has entailed some delay in publication, it was felt that this would add to the standard of contributions and increase the scientific acceptability of the papers.

The other three sections, the Extended Abstracts, Program Abstracts and Workshop Summaries, have not been subjected to external review and are published as received.

In order to maintain uniformity of script, all the papers and abstracts were retyped. However, it was not possible to maintain uniformity in the usage of symbols, such as mg/L or mg L^{-1}, because various papers used different notations, and to bring consistency here would have entailed re-doing all the figures and tables.

Proceedings of this and earlier Workshops may be obtained from the following address:

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MICROBIAL ACTIVITY IN SEDIMENTS FROM ACIDIC ONTARIO LAKES

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BAKER, M.D., P.T.S. WONG, W.E. INNISS, and C.I. MAYFIELD. 1981. Microbial activity in sediments from acidic Ontario lakes. Can. Tech. Rep. Fish. Aquat. Sci.

The microbial activity in sediments from three acidic Ontario lakes was examined. The sediment of Big Turkey Lake was pH 6.2, while Plastic Lake and Little Turkey Lake had sediment pH values of 5.8 and 5.7, respectively. These two lake sediments also had slightly higher organic matter and carbohydrate contents. On a comparative basis, the sediment of Plastic Lake contained the lowest number of aerobic, heterotrophic bacteria. Measurements of microbial activity suggested that the microorganisms in the Plastic Lake and Little Turkey Lake sediments were less active than those in the Big Turkey Lake sediment.

Studies were conducted to determine the effect of pH on the microbial activity in the Plastic Lake sediment. The results revealed that both oxygen uptake and the mineralization of various organic substrates were reduced in acidic conditions.

Key words: Microbes, sediment, lakes, acidic, low pH.

BAKER, M.D., P.T.S. WONG, W.E. INNISS, and C.I. MAYFIELD. 1981. Microbial activity in sediments from acidic Ontario lakes. Can. Tech. Rep. Fish. Aquat. Sci.

L'activité microbienne a été examinée dans les sédiments de trois lacs acides. Le pH des sédiments du lac Big Turkey est de 6.2 alors que ceux des lacs Plastic et Little Turkey sont respectivement de 5.8 et 5.7. Les sédiments de ces 2 derniers lacs ont aussi un peu plus de matière organique et d'hydrates de carbone. Comparativement, les sédiments du lac Plastic contiennent la plus petite quantité de bactéries hétérotrophes et aérobiques. L'évaluation de l'activité microbienne suggeste que les microorganismes présents dans les sédiments des lacs Plastic et Little Turkey sont moins actifs que ceux présents dans les sédiments du lac Big Turkey.

Des études ont été menées pour déterminer l'effet du pH sur l'activité microbienne dans les sédiments du lac Plastic. Les résultats révèlent que l'absorption d'oxygène et la minéralisation de divers substrats organiques sont réduites dans des conditions acides.

INTRODUCTION

Acid precipitation has resulted in a decrease in the pH of hundreds of poorly buffered lakes in Scandinavia and North America (Beamish 1974; Dickson 1975; Gjessing et al. 1976; Likens and Bormann 1974; Likens et al. 1979; National Research Council Canada 1981; Oden 1976; Wright and Gjessing 1976). The most noticeable effect of acidification has been the loss of entire populations of freshwater fish (Beamish 1974; Beamish and Harvey 1972; Leivestad et al. 1976; Schofield 1976). However, acid precipitation has been shown to affect all trophic levels (Hendrey et al. 1976; Leivestad et al. 1976).

In the aquatic ecosystem the decomposition of organic materials is accomplished mainly through the activities of microorganisms. However, in acidic lakes, organic matter accumulates on the sediment surface presumably due to a decrease in these microbial decomposition processes (Grahn and Hultberg 1974; Grahn et al. 1974). A reduction in the microbial activity in acidified lakes could have adverse effects on the entire aquatic ecosystem. This report examines the effect of acidification on the activity of sediment microorganisms.

MATERIALS AND METHODS

Sediment

Sediment samples were obtained from oligotrophic lakes located in two areas of Ontario. The lakes sampled were Plastic Lake (latitude 45°11'N; longitude 78°50'W) near Dorset, Ontario, Big Turkey Lake (latitude 47°03'N; longitude 84°26'W) and Little Turkey Lake (latitude 47°03'N; longitude 84°25'W), both located approximately 50 km north of Sault Ste. Marie, Ontario. Sediments were collected with an Eckman grab sampler, transported in ice and stored in the laboratory at 4°C.

Several characteristics of each of the lake sediments were determined. The pH was measured under an aerobic gas phase with stirring. Moisture contents were determined by oven-drying replicate samples of each of the sediments at 60°C for 24 h. Carbohydrate content was measured with the modified phenol-sulphuric acid method of Liu et al. (1973) using Nobel agar as the carbohydrate standard. Total combustible organic matter was estimated by ashing at 600°C for 24 h. The number of viable aerobic heterotrophic bacteria in each lake sediment was enumerated by a spread-plating technique using an agar medium consisting of nutrient broth, 5 g; glucose, 1 g; yeast extract, 1 g; agar, 20 g and distilled water, 1000 mL. Agar plates were incubated at 0°C and 20°C for 28 and 7 days, respectively, after which the total number of bacteria growing at each temperature was determined.

Oxygen Uptake

Oxygen uptake studies were conducted to determine the degree of microbial activity present within each lake sediment. Oxygen uptake was measured with shaking at 20°C using a Gilson differential respirometer (Gilson Medical Electronics Inc., Middleton, Wis.). All experiments were completed within 24-48 h of obtaining the sediments using 4 mL of sediment in Warburg flasks. Using standard manometric procedure the uptake of oxygen was measured for

60 min.

Sediment pH values in lakes subject to acid precipitation may vary so an experiment was conducted whereby the sediment of Plastic Lake was artificially adjusted to different pH values. Sediment-lake water slurries (1.5 parts wet weight sediment: 1 part lake water) were gently stirred using a magnetic stirring bar. After equilibration at 20°C the pH of each separate slurry was adjusted with either 1 or 2 N sulphuric acid or 1 or 2 N sodium hydroxide to final pH values of 4.5, 5.5, 6.5 and 7.5. Oxygen uptake was determined as described above. Three replicates were used for each treatment. All values were converted to μL of oxygen consumed g^{-1} sediment dry weight.

Mineralization of ^{14}C-labelled Organic Compounds

Mineralization experiments similar to those of Harrison et al. (1971) were conducted in order to obtain more information on the extent of microbial activity in the lake sediments and also to determine the ability of the sediment microorganisms to degrade organic matter. In these studies duplicate 50 mL hypo-vials were used for each treatment plus a control sample containing 2 mL of 1 N sulphuric acid to correct for blank activity. The vials contained 0.1 μCi of uniformly-labelled ^{14}C-glucose (specific activity of 260 mCi $mMole^{-1}$). The final volume in each vial was made up to 2 mL by the addition of distilled water. Sediment from each lake was allowed to equilibrate with stirring for 2 h at 20°C. After equilibration, 4 mL of each sediment was added to the appropriate vials and each vial was sealed with a rubber serum cap which held a plastic cup and rod assembly (Kontes Glass Co., Vineland, N.J.). The cup assembly contained an accordian-folded piece of Whatman #1 filter paper (2.5 x 5.0 cm). Each serum cap was sealed with silicone rubber sealant. Mineralization was allowed to proceed at 20°C and after the appropriate times, 1 mL of 1 N sulphuric acid was injected into the sediment systems. The vials were left for 1 h with frequent agitation after which 0.15 mL of β-phenethylamine was injected onto the filter paper to absorb all released $^{14}CO_2$. The vials were left for an additional 1 h and then the filters were removed and placed in scintillation vials containing 10 mL of PCS liquid scintillation fluid (Amersham-Searle Ltd.). The radioactivity was measured with a Beckman Model LS8100 liquid scintillation counter. The channels-ratio method was used to correct for quenching and all samples were corrected for control activity. The trapping efficiency of the β-phenethylamine was measured by releasing a known amount of $^{14}CO_2$ from $NaH^{14}CO_3$ with 1 N sulphuric acid and was between 95 and 100%.

The mineralization of ^{14}C-glucose was also measured in Plastic Lake sediment-lake water systems adjusted to pH values of 4, 5, 6 and 7 with either 1 or 2 N sulphuric acid or 1 or 2 N sodium hydroxide. Mineralization was allowed to proceed for 10 min at 20°C. In other experiments the mineralization of the uniformly-labelled substrates ^{14}C-glucose, ^{14}C-glycine (specific activity of 112 mCi $mMole^{-1}$) and ^{14}C-glutamic acid (specific activity of 265 mCi $mMole^{-1}$) was compared. In this study sediment-lake water systems were adjusted to pH values of 4 and 7 using sulphuric acid and sodium hydroxide. Each hypo-vial contained 0.1 μCi of the appropriate ^{14}C-labelled compound. Mineralization was allowed to proceed for 10 min at 20°C. All other procedures for the mineralization experiments were the same as described previously.

RESULTS AND DISCUSSION

Acid precipitation primarily affects watersheds which are located in areas that are highly resistant to chemical-weathering processes (Wright and Gjessing 1976). Many Ontario lakes which are located in such areas are, or are becoming, more acidic (Beamish and Harvey 1972; Dillon et al. 1978; Scheider et al. 1978). The lakes studied in this investigation are located within areas that are extremely sensitive to, and receive inputs of acidic precipitation (Dillon et al. 1978; Likens et al. 1979; Scheider et al. 1978). Measurement of the sediment pH (Table 1) showed that they were all acidic. Plastic Lake and Little Turkey Lake were slightly more acidic with pH values of 5.8 and 5.7, respectively. The pH of the Big Turkey Lake sediment was higher at 6.2. It is known that acidic lakes often contain high concentrations of organic matter, including leaf litter, which accumulates on the sediment surface due presumably to a decrease in the microbial decomposition processes (Grahn and Hultberg 1974; Grahn et al. 1974). Both the Plastic Lake and Little Turkey Lake sediments contained higher concentrations of carbohydrate and organic matter. The Plastic Lake sediment also contained the lowest number of aerobic heterotrophic bacteria at both 0°C and 20°C.

Table 1. Characteristics of the sediments

Lake	pH	Moisture content (%)	Carbohydrate content[1]	Organic matter[1]	Bacteria g^{-1} sediment dry weight	
					0C	20C
Plastic	5.8	90.6	86.8	385.6	3.1×10^4	9.5×10^5
Little Turkey	5.7	94.4	58.0	420.8	5.2×10^6	1.0×10^7
Big Turkey	6.2	88.4	36.5	336.7	8.2×10^6	1.8×10^7

[1] mg g^{-1} sediment dry weight

The oxygen uptake rates of these sediments (Fig. 1) possibly suggested that the Plastic Lake and Little Turkey Lake sediments were less microbiologically active. At the end of the 60-min incubation, the oxygen uptake for the Little Turkey Lake and Plastic Lake sediments was only 36 and 27%, respectively, of that in the Big Turkey Lake sediment.

Studies involving the mineralization of ^{14}C-glucose showed a similar pattern (Fig. 2).

Although a large number of factors, including pH, control the growth and

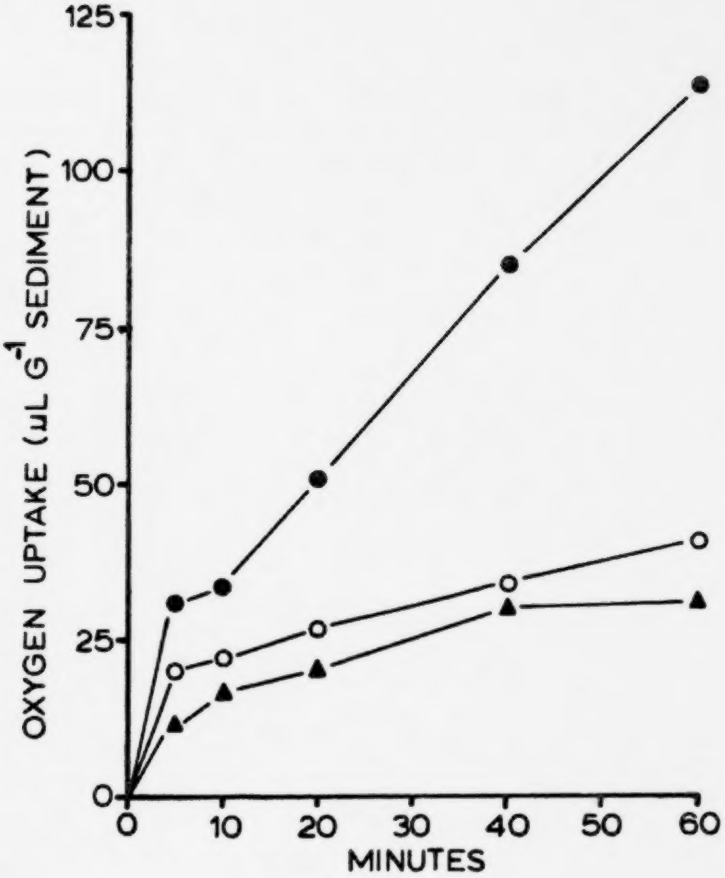

Fig. 1. Microbial oxygen uptake in acidic lake sediments. Plastic Lake (▲); Little Turkey Lake (○); Big Turkey Lake (●).

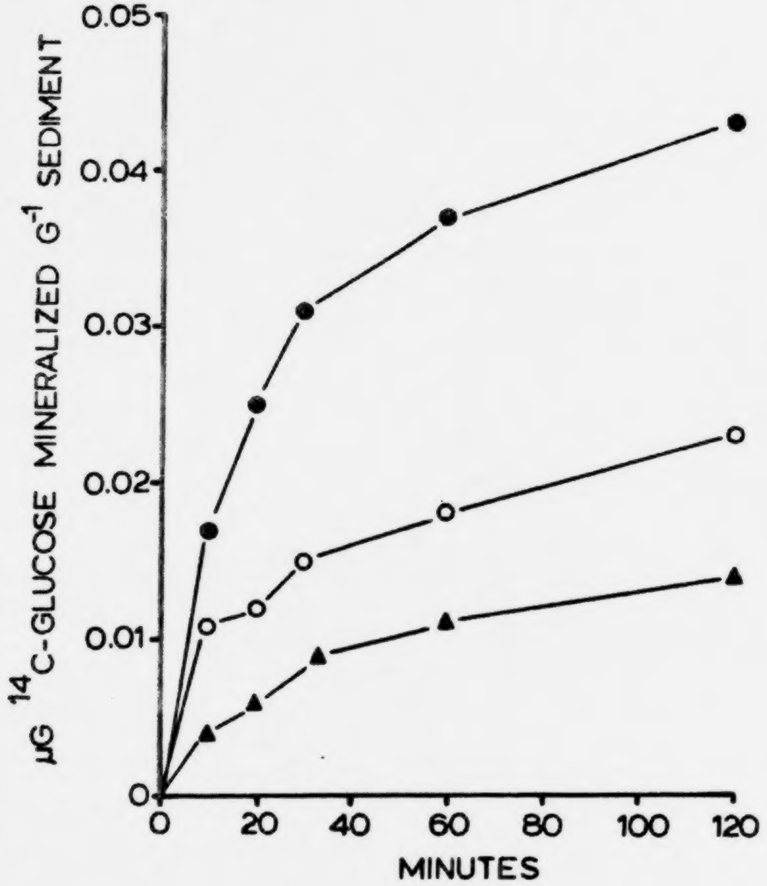

Fig. 2. Mineralization of ^{14}C-glucose in acidic lake sediments. Plastic Lake (▲); Little Turkey Lake (○); Big Turkey Lake (●).

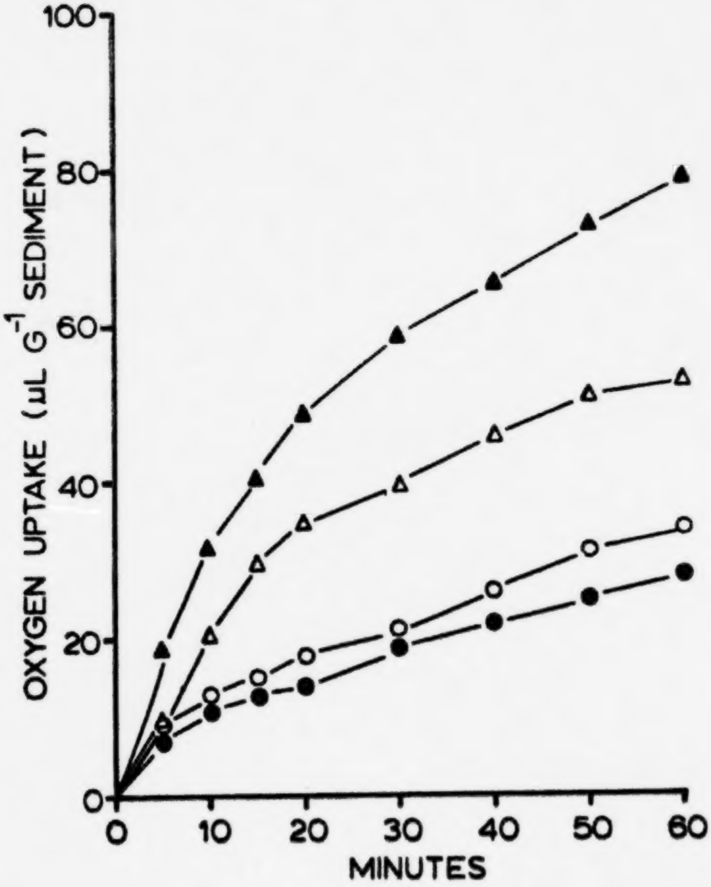

Fig. 3. Effect of pH on the microbial oxygen uptake in the Plastic Lake sediment. Treatments: pH 4.5 (●); pH 5.5 (○); pH 6.5 (△); pH 7.5 (▲).

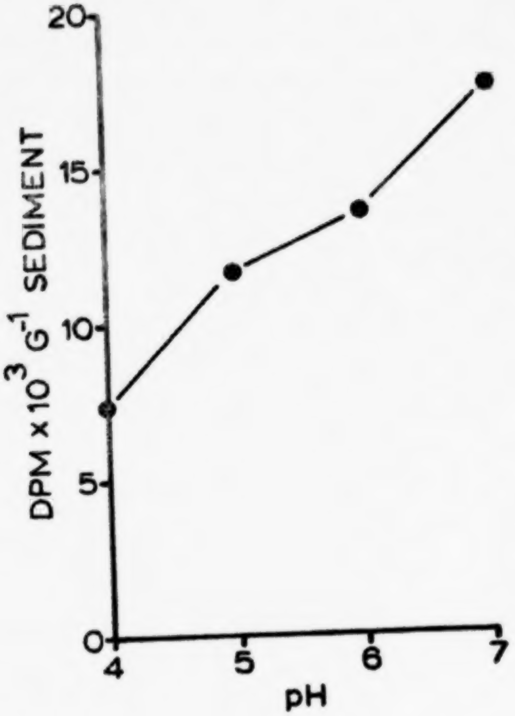

Fig. 4. Effect of pH on the mineralization of ^{14}C-glucose.

activity of microorganisms, the results of these studies suggest that the microorganisms in both the Plastic Lake and Little Turkey Lake sediments were less active. The apparent reduction in microbial activity (possibly due to the low sediment pH) may have contributed to the higher concentrations of both organic matter and carbohydrate in these two lake sediments.

Oxygen uptake was measured for sediment adjusted to pH values from 4.5 to 7.5 (Fig. 3). Considering the first 10 min of incubation at sediment pH values of 7.5, 6.5, 5.5 and 4.5, the oxygen uptake rates were 3.2, 2.1, 1.3 and 1.1 μL of oxygen consumed per g of sediment dry weight per min, respectively. Total oxygen uptake over the 60-min incubation also decreased at low pH. If the total amount of oxygen consumed at pH 7.5 represents 100%, then the total oxygen consumption at pH values of 6.5, 5.5 and 4.5 was reduced by 33, 57 and 65%, respectively.

The effect of pH on the mineralization of ^{14}C-glucose by the Plastic Lake sediment showed similar results (Fig. 4). At pH values of 4, 5 and 6, glucose mineralization was reduced 59, 34 and 22%, respectively, compared to pH 7.

In additional experiments, comparison of the mineralization of ^{14}C-glucose, ^{14}C-glycine and ^{14}C-glutamic acid showed that mineralization was reduced 32, 61 and 69%, respectively, at pH 4 as compared to pH 7.

The results of the altered pH experiments showed that acidic pH levels decreased the activity of the sediment microorganisms. The low oxygen uptake and reduced ability of mineralize the simple organic compounds used suggests that recalcitrant materials found in aquatic systems will persist for even longer periods of time. The reduction in organic decomposition would alter the exchange and cycling of nutrients and this could have a deleterious effect on the higher trophic levels.

ACKNOWLEDGMENTS

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THE DYNAMICS AND PERSISTENCE OF THE HERBICIDE AQUAKLEEN® IN SMALL ARTIFICIAL PONDS AND ITS IMPACT ON NON-TARGET AQUATIC MICROFLORA AND MICROFAUNA

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BIRMINGHAM, B.C., M. THORNDYKE, and B. COLMAN. 1981. The dynamics and persistence of the herbicide Aquakleen® in small artificial ponds and its impact on non-target aquatic microflora and microfauna. Can. Tech. Rep. Fish. Aquat. Sci.

Outdoor artificial ponds planted with *Myriophyllum spicatum* were treated with Aquakleen granules (20% butoxyethanol ester of 2,4-D: BEE) at the rate of 23 kg active ingredient ha^{-1}. *M. spicatum* had completely collapsed 5 days after application and filamentous green algae had invaded the ponds by 21 days. Significant decreases in dissolved O_2 level and pH, and increases in dissolved inorganic carbon occurred 7 days after treatment. No dissolved nutrient increase was detected on decay of the weeds and unicellular algae did not bloom. Zooplankton numbers declined in the 14 days following treatment due to the reduction in number of rotifers and ostracods.

2,4-D residues in pond water rapidly declined to 1.0 mg L^{-1} after 85 days and 0.2 mg L^{-1} after 178 days. Residues in plant material dropped to 10 - 20 μg g dry wt^{-1} after 70 days while in sediment they were negligible after 50 days. This study indicates that 2,4-D degrades slowly in water under Canadian climatic conditions, but that its impact is due to the removal of the weed-bed ecosystem.

Key words: *Myriophyllum spicatum*; 2,4-D, butoxyethanol ester; aquatic ecosystem; Canadian climatic conditions.

BIRMINGHAM, B.C., M. THORNDYKE, and B. COLMAN. 1981. The dynamics and persistence of the herbicide Aquakleen® in small artificial ponds and its impact on non-target aquatic microflora and microfauna. Can. Tech. Rep. Fish. Aquat. Sci.

Myriophyllum spicatum a été planté dans des étangs artificiels extérieurs. Ceux-ci furent traités avec des granules d'Aquakleen (20% butoxyethanol ester of 2,4-D: BEE), 23 kg d'ingrédients actifs ha^{-1}. *M. spicatum* s'effondre complètement 5 jours après l'application et des algues filamenteuses envahissent les étangs en dedans de 21 jours. Après 7 jours de traitement, le niveau d'oxygène dissous et le pH diminuent de façon significative et la quantité de carbone inorganique dissoute augmente significativement. La décomposition de *Myriophyllum* ne produit pas d'augmentation dans la quantité d'éléments nutritifs dissous et les algues unicellulaires n'ont pas d'explosion de population. La quantité de zooplancton décline dans les 14 jours suivants le traitement et ceci est dû à la diminution du nombre de rotifères et d'ostracées.

Les résidus de 2,4-D dans l'eau des étangs diminuent rapidement et atteignent 1.0 mg ℓ^{-1} après 85 jours et 0.2 mg ℓ^{-1} après 178 jours. Les résidus mesurés dans les plantes tombent à 10 - 20 µg matière sèche^{-1} après 70 jours tandis que, dans les sédiments, ils sont négligeables après 50 jours. Cette étude indique que le 2,4-D se dégrade lentement dans l'eau, sous l'influence du climat canadien, mais que son impact est dû à l'élimination de l'écosystème des plantes aquatiques.

INTRODUCTION

In Canada, Aquakleen® (20% butoxyethanol ester of 2,4-D on attaclay particles) is the only 2,4-D herbicide registered for control of Eurasian water milfoil (*Myriophyllum spicatum* L.), water lilies and cattails in the still waters of ponds and lakes. The impact of using Aquakleen to control aquatic nuisances like Eurasian water milfoil in Canadian aquatic ecosystems had not been adequately investigated when this present study was initiated. Little published information exists on the rate of breakdown of the butoxyethanol ester (BEE), its uptake by aquatic plants, its toxicity to non-target aquatic flora and fauna and its persistence in the biotic and abiotic components of the aquatic ecosystem under Canadian conditions.

Earlier studies of the fate of BEE in aquatic ecosystems (Smith and Isom 1967; Frank and Comes 1967) and its impact on target and non-target organisms (Elliston and Steward 1977; Saunders 1970; Sigmon 1979a, 1979b; Butler 1965; Meehan et al. 1974; Dodson and Mayfield 1979) have either been carried out in the laboratory or in parts of the world with much warmer climates.

In this study, shallow, weed-infested artificial ponds on York University campus were used to investigate:

A. the persistence, degradation and fate of BEE under Canadian conditions, and

B. the impact of Aquakleen treatment on water chemistry, aquatic flora (phytoplankton, filamentous green algae and water weeds), and aquatic microfauna (zooplankton and insect larvae).

This work was undertaken to improve the data-base relative to environmental assessment of Aquakleen under Canadian conditions.

MATERIALS AND METHODS

CONSTRUCTION AND TREATMENT OF ARTIFICIAL PONDS

The artificial ponds were one meter deep, polyethylene-lined depressions measuring 2.2 m x 2.2 m. The bottom of the ponds was covered with approximately 15 cm of virgin loam, filled with approximately 5000 L tap water and planted with approximately 50 *Myriophyllum spicatum* plants per pond. Within 2 months a dense growth of *Myriophyllum* had developed in all ponds.

On August 24, 1980, Aquakleen granules at the rate of 23 kg active ingredient ha^{-1} were applied evenly over the surface of 3 ponds. Three other ponds were used as controls.

CHEMICAL ANALYSIS

Subsurface measurements of pH, dissolved oxygen and temperature were performed *in situ*. At least 3 random vertical water samples were collected for each pond, pooled in 1-gal glass bottles and immediately brought to the laboratory for analysis. The dissolved inorganic carbon (DIC) content of unfiltered pond water was determined using a gas chromatograph (Birmingham and Colman 1979). The material collected after filtering 200 mL to 1 L of pond water through Whatman GF/C glass fibre filters was extracted and analysed fluorimetrically for chlorophyll *a* and phaeopigments (Strickland and Parsons 1972). Fifty mL of filtered pond water were digested according to Nicholls (1975) and analysed for total soluble phosphorus using the ascorbic acid method (Standard Methods 1975) and total soluble organic nitrogen using the indophenol method (Scheiner 1976). Twenty-five mL aliquots of undigested filtered pond water were also directly analysed for soluble organic nitrogen. Twenty-five mL aliquots of filtered pond water were analysed for nitrate following hydrazine sulfate reduction to nitrite (Kamphake et al. 1967). Nitrite was measured following diazotization according to Standard Methods (1975).

RESIDUE ANALYSIS

Three-hundred to 500 mL aliquots of filtered pond water were acidified to less than pH 2 by addition of H_2SO_4 and extracted 3 times with 50 mL portions of methylene chloride. The organic extracts were combined, dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was methylated with diazomethane and injected into the gas chromatograph.

One-hundred to 500 g portions of wet sediment (subsampled for dry wt determination) were suspended in 400 mL distilled water and acidified to less than pH 2 with H_2SO_4 and extracted 3 times with 100 mL portions of ether. Extracts were combined and treated as described above. Plant material was blended at high speed in 200 mL to 500 mL distilled water. The slurry was acidified, extracted and derivatized as described for the sediment samples.

The gas chromatograph used was a Barber-Colman series 5000 equipped with a flame ionization detector. The 2 m by 6 mm i.d. glass column was packed with a 50:50 mixture of 10% OV-11 and 10% OV-17 on Chromosorb W, H.P., 80-100 mesh. Nitrogen, zero gas, was carrier gas at a flow rate of 100 mL min^{-1}. Operating conditions were as follows: injection temperature - $25^{o}C$; column temperature, isothermal at $190^{o}C$ for 5 min then programmed to $310^{o}C$ over the next 10 min; detector temperature - $315^{o}C$. Hydrogen and compressed air flow rates were 22 and 195 mL min^{-1}, respectively. Under these conditions, retention times for the methyl and butoxyethanol esters of 2,4-D were 7 and 11 min, respectively. The amounts of each ester in the samples were obtained by comparison with standard curves.

PHYTOPLANKTON ANALYSIS

Eight-hundred mL unfiltered pond water was preserved with Lugol's iodine.

Phytoplankton samples were concentrated by sedimentation and the algae counted and identified in an inverted microscope fitted with a condenser.

ZOOPLANKTON ANALYSIS

Zooplankton were sampled by taking at least 3 random verticle 50 cm "sweeps" using a 14 cm diameter dip net (76 μm mesh). The contents of these "sweeps" for each pond were pooled and preserved in 4% formalin. Zooplankton samples were identified and enumerated directly under low or high magnification using a Wards zooplankton counting wheel.

RESULTS

Water temperature dropped from about 25°C at treatment to freezing temperatures within 2 months and the ponds were frozen for the remainder of the study. This enabled us to follow 2,4-D dynamics under Canadian climatic conditions.

The 2,4-D residue data is shown in Figure 1. Each point is the mean value for the 3 treated ponds. The initial level of the ester in water was very low (0.16 mg L^{-1} one day after application) and it decreased to less than 0.01 mg L^{-1} within 15 days. This rapid disappearance of the ester is a reflection of both its low water solubility, its uptake by the water weeds and its rapid hydrolysis to the free acid form of 2,4-D (Dodson and Mayfield 1979). The level of 2,4-D acid in water rose to 3 mg L^{-1} over the first 2 weeks and then gradually decreased to about 1 mg L^{-1} after 85 days. When the ice thawed about 0.2 mg L^{-1} of 2,4-D was present in water after 180 days.

The ester persisted much longer in the sediment. Since the Aquakleen granules sank to the bottom of the ponds, this may represent either direct sampling of undegraded granules or the sorption of the ester by the organic component of the sediments. Ester concentration in the mud rose to 1.7 μg g^{-1} dry wt one week after treatment and then rapidly decreased to 0.1 μg g^{-1} dry wt over the next 6 weeks. Initial levels of 2,4-D acid in the sediment were quite high (mean value of 6.7 μg g^{-1} dry wt one day after treatment) and then dropped to the levels found in the water column.

Combined ester and acid residues in the plant material were 10- to 100-fold higher and this curve corresponds to the concentration scale of the right hand of Figure 1. Considerable bioaccumulation of 2,4-D by plant material was observed in the 2 weeks following treatment. A mean maximum of 206 μg 2,4-D g^{-1} dry wt was observed 9 days after treatment. This represents 2,4-D taken up mainly by *Myriophyllum* because residue levels dropped to 10-20 μg 2,4-D g^{-1} dry wt after this period. This lower accumulation of 2,4-D is related to the transition from water weed to filamentous green algae that occurred in these ponds.

The natural succession observed in the ponds was one of increasing infestation of the water weeds by filamentous green algae. These algae developed either as floating "balls" or attached to the weeds, and were mainly a mixture of *Rhizoclonium* sp. and *Oedogonium* sp. Ten to 50 percent coverage of the control ponds by filamentous green algae occurred 4 to 7 weeks after treatment

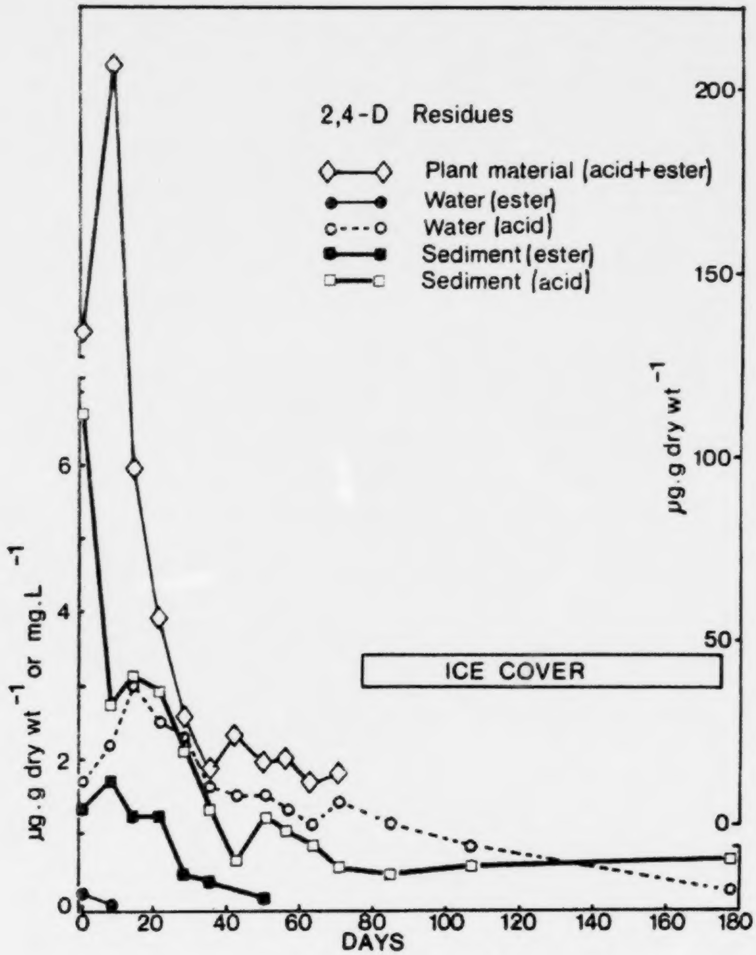

Fig. 1. Mean 2,4-D residues in water, sediment and plant material of the treated ponds.

started. In treated ponds, *Myriophyllum* collapsed to the bottom of the ponds, 5 days after application. Filamentous green algae developed on the dead weeds and had completely invaded the treated ponds 3 weeks after treatment.

Death of the water weeds following application of Aquakleen had significant effects on the water chemistry of the treated ponds. Levels of both dissolved oxygen and pH dropped significantly ($P < 0.05$, Student's *t*-test) in the treated ponds (Fig. 2). On the other hand, the total dissolved inorganic carbon level rose in the treated ponds. These changes in pond water chemistry were caused by the loss of the actively photosynthesizing water weeds. The rise in total dissolved inorganic carbon in treated ponds was not quite significant at the 5% level. However, dramatic differences in the calculated free CO_2 level (based on the DIC and pH data) between the 2 sets of ponds are evident (Fig. 3). In control ponds, calculated free CO_2 levels rarely exceeded 0.1 $\mu M\ CO_2\ L^{-1}$ and this is a strong indication of the high photosynthetic efficiency of *Myriophyllum*. In treated ponds, death and decay of the water weeds lead to large increases in the calculated free CO_2 level. These free CO_2 levels exceeded the calculated free CO_2 equilibrium level and suggest high rates of respiration associated with weed decay.

There was no evidence for massive release of dissolved nutrients in treated ponds following death and decay of the water weeds. No significant differences in the level of dissolved organic nitrogen, total soluble phosphorus or nitrate were observed between control and treated ponds (Fig. 4). Levels of nitrate were very low ($< 10\ \mu g\ L^{-1}$ during the month following treatment. This suggests that the ponds were nitrate-limited. Low levels of nitrate especially during late summer and fall appears to be a characteristic of shallow enclosed bodies of water (Peverly et al. 1979; O'Brien et al. 1974; Haertel 1976) and are related to biological utilization and/or the denitrification properties of the sediments (Keeney 1973).

This nitrate limitation is reflected in the fact that no phytoplankton blooms were observed and the ponds remained transparent throughout the study. The phytoplankton consisted of 25 species of *Chlorphyta*, 2 species of *Cryptophyta*, 3 species of *Cyanophyta*, and 9 species of *Chryophyta*. The mean total phytoplankton numbers for both sets of ponds are shown in Figure 5. The mean population peak observed in the control ponds was caused by a large population of the extremely small blue-green alga, *Rhabdoderma irregulare*. In terms of biomass this population peak is only apparent since chlorophyll *a* readings in both sets of ponds were less than $10\ \mu g L^{-1}$. Phaeophytin values were greater than 50% suggesting heavy grazing by the zooplankton.

Mean total zooplankton numbers for control and treated ponds are shown in Figure 6. Zooplankton in the ponds consisted of rotifers, cyclopoid copepods, cladocerans, ostracods and insect larvae, mainly *Chaoborus* larvae. Zooplankton numbers were higher in the control ponds in the 2 weeks following treatment. This was mainly due to population peaks of rotifers and ostracods which were not observed in the treated ponds during this period. Population densities then became similar in both sets of ponds and subsequently declined in the treated ponds. This decline in the treated ponds was mainly due to the virtual absence of rotifers and ostracods and a substantial reduction in copepod and cladocera numbers.

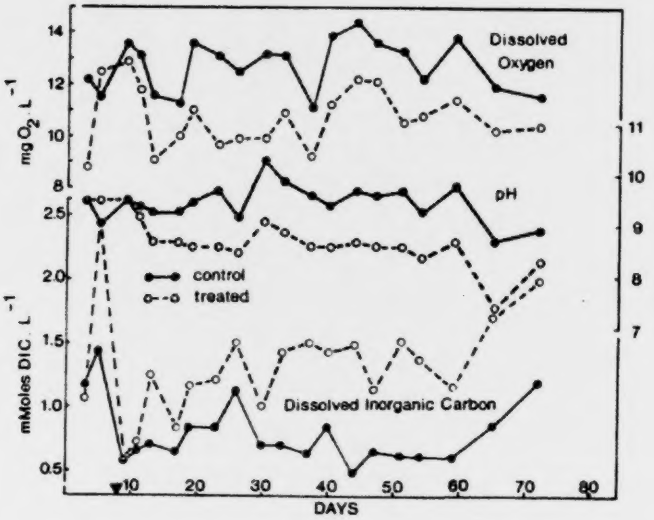

Fig. 2. Mean Dissolved Oxygen, pH and Dissolved Inorganic Carbon values in treated and control ponds (▼ treatment date).

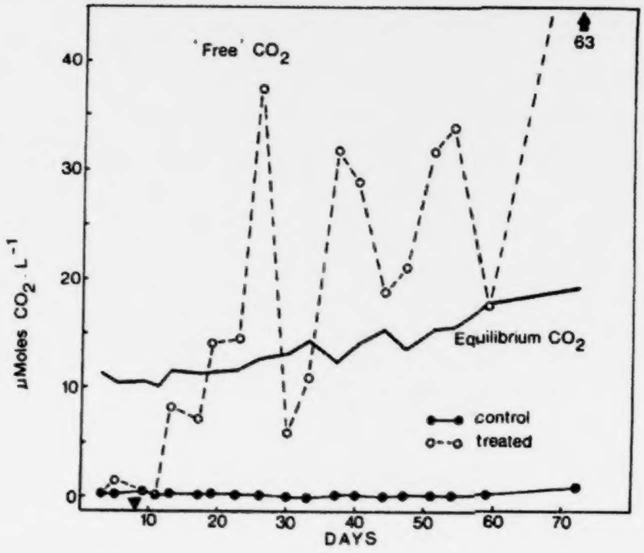

Fig. 3. Mean calculated free CO_2 values in treated and control ponds.

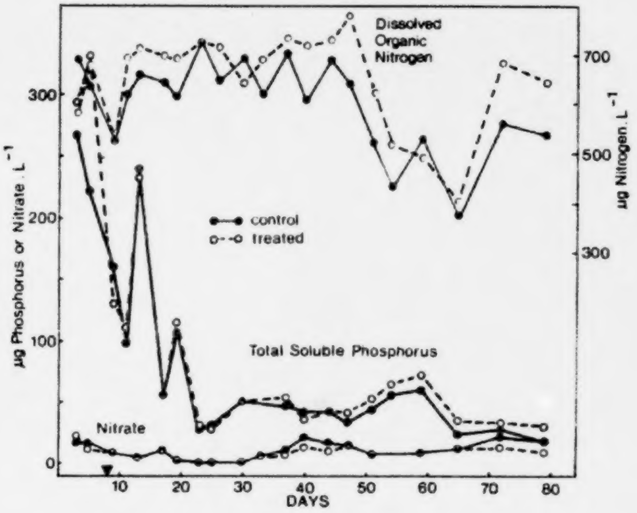

Fig. 4. Mean Dissolved Organic Nitrogen, Total Soluble Phosphorus and Nitrate values in treated and control ponds.

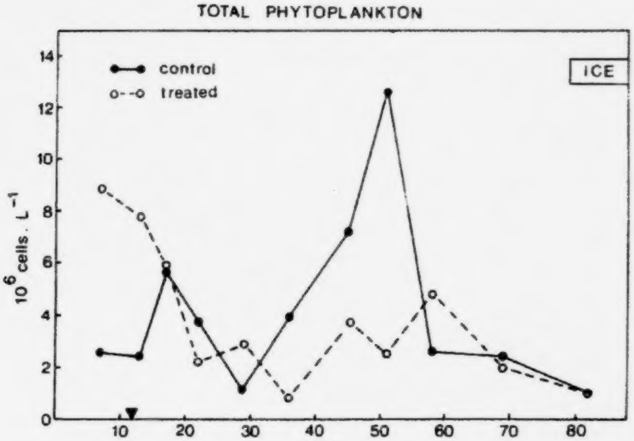

Fig. 5. Mean total phytoplankton numbers in control and treated ponds.

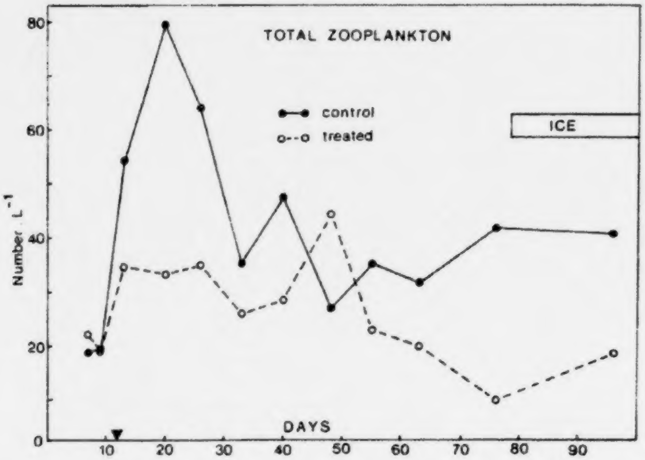

Fig. 6. Mean total zooplankton numbers in control and treated ponds.

CONCLUSION

There are two major aspects of this investigation using weed-infested artificial ponds; first, the persistence and fate of the 2,4-D ester in these enclosures; and second, the impact of weed removal on pond water chemistry and non-target organisms.

The information in Figure 1 on the persistance and fate of 2,4-D, clearly shows that in shallow, weed-infested waters with a high plant-to-water volume ratio there is considerable accumulation of the lipid-soluble 2,4-D ester by plant material. The 2,4-D ester also appears to persist in the sediment presumably adsorbed to organic matter (and Aquakleen granules on the sediment surface). Only trace amounts of the ester were found in the water column. It can be seen that after 6 weeks, most of the ester had dissipated, however, the ester was hydrolysed to the free acid form and persisted as such in the water and sediments for up to 180 days. This is a significant finding of this study and suggests that during Canadian winter conditions (even in southern Ontario) 2,4-D residues can persist in enclosed aquatic systems. This confirms earlier laboratory studies that indicated the persistence of 2,4-D in cold water systems (De Marco et al. 1967) and this persistence may be due to reduced microbial activity at low temperatures (Nesbitt and Watson 1980).

It can be seen that loss of the actively photosynthesizing water weeds caused significant changes in the levels of dissolved oxygen, free CO_2 and pH (Fig. 2). Death and decay of these water weeds did not lead to massive release of dissolved nutrients. However, significant changes in the ecosystem structure had occurred.

It is evident that difficulties arise when trying to separate direct toxic effects of the herbicide from indirect environmental effects resulting from ecosystem changes, i.e., loss of weed habitat and ensuing changes in water chemistry. No mass mortality of zooplankton was observed in this study though removal of the weed habitat appears to be deleterious to rotifers and ostracods. 2,4-D appears to have little impact on phytoplankton algae and, in fact, the *Myriophyllum* nuisance was replaced by another aquatic nuisance, the filamentous green algae.

In terms of aquatic toxicology and risk assessment, artificial ponds appear to be a halfway house between *in vitro* laboratory studies and direct field testing. They are a complex artificial test system in which the initial test conditions are reasonably controllable. They are convenient to sample and any number of replicates can be constructed. They also prevent release of the chemical being tested into the environment. The results of this study suggest that 2,4-D can persist in shallow enclosed bodies of water under Canadian climatic conditions and that the impact of 2,4-D is mainly a consequence of its destruction of the weed-bed ecosystem.

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POTENTIEL DE BIOACCUMULATION DE SUBSTANCES TOXIQUES D'EAUX RESIDUAIRES INDUSTRIELLES A L'AIDE D'UN ESSAI UTILISANT DES ALGUES ET DES BACTERIES

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BLAISE, C., B. SKA, G. SABATINI, N. BERMINGHAM, et R. LEGAULT. 1981. Potentiel de bioaccumulation de substances toxiques d'eaux résiduaires industrielles à l'aide d'un essai utilisant des algues et des bactéries. Can. Tech. Rep. Fish. Aquat. Sci.

Dans le cadre d'une étude à long terme orientée ultimement vers la spéciation de la toxicité, nous avons élaboré une méthode d'analyse relativement simple et rapide ayant pour but la détection du potentiel de bioaccumulation de substances toxiques provenant des eaux usées. La technique employée a fait appel aux propriétés accumulatrices de l'algue *Selenastrum capricornutum* ainsi qu'au système de détection de toxicité Microtox de Beckman, lequel exploite les caractéristiques bioluminescentes de la bactérie *Photobacterium phosphoreum*. La méthode employée, les plus récents résultats, ainsi que l'orientation et l'application futures de ces travaux, sont systématiquement exposés.

Mots clés: Toxicité; bioaccumulation; algues; *Selenastrum capricornutum*; bactéries; Microtox.

BLAISE, C., B. SKA, G. SABATINI, N. BERMINGHAM, et R. LEGAULT. 1981. Potentiel de bioaccumulation de substances toxiques d'eaux résiduaires industrielles à l'aide d'un essai utilisant des algues et des bactéries. Can. Tech. Rep. Fish. Aquat. Sci.

Within the framework of a long-term study oriented ultimately toward the speciation of toxicity, we have developed a relatively simple and rapid method of analysis designed to monitor the bioaccumulation potential of wastewaters. The technique employed uses the bioaccumulative properties of the alga *Selenastrum capricornutum* as well as the Beckman Microtox toxicity monitoring system, which exploits the bioluminescent properties of the bacterium *Photobacterium phosphoreum*. The methodology employed, the most recent results, and the orientation and future application of this work are systematically discussed.

INTRODUCTION

Diverses techniques de bioanalyse ont vu le jour depuis l'époque héroique où le test de la toxicité létale aiguë utilisant la truite arc-en-ciel comme indicateur biologique régnait incontestablement seul dans le cadre d'études sur la toxicité en milieu aquatique. Celui-ci constitue toujours, à l'heure actuelle, l'unique moyen biologique, du point de vue juridique, par lequel on évalue la toxicité des différents rejets liquides dans l'environnement au Canada (Environnement Canada, 1980). Depuis la dernière décennie, cependant, les outils biologiques, pour fins d'évaluation de la toxicité, n'ont cessé de se multiplier et de se raffiner afin d'être en mesure de répondre à des besoins particuliers. La période présente est nettement caractérisée par l'importance attachée aux aspects sublétaux, chroniques, persistants, bioaccumulateurs et mutagéniques de la toxicité en milieu aquatique et par l'intérêt marqué envers les essais microbiologiques (algues, protozoaires, bactéries, tissus cellulaires).

Dans ce contexte, nous avons mis l'accent sur la mise au point d'un test relativement simple, rapide et peu coûteux, capable de détecter le potentiel de bioaccumulation de matières toxiques de tout rejet liquide dans l'environnement. Il est apparu profitable, pour atteindre cet objectif, de faire appel aux propriétés accumulatrices de l'algue *Selenastrum capricornutum*, ainsi qu'au système de détection de toxicité Microtox de Beckman lequel exploite les caractéristiques bioluminescentes de la bactérie *Photobacterium phosphoreum*.

Le *biomonitoring* exploitant la capacité des algues à accumuler diverses composantes inorganiques (Friant and Koerner, sous presse) et même organiques (Sheldon and Hites, 1978) du milieu aquatique a déjà fait ses preuves et, en soi, ce concept n'est pas nouveau. Néanmoins, ce genre d'étude nécessite la détermination d'une quantité parfois fastidieuse de paramètres chimiques, lesquels détermineront les substances minérales et/ou organiques qui se sont accumulées dans les algues en rapport avec leurs teneurs respectives dans le cours d'eau étudié. Le coût prohibitif d'un tel support chimique constitue un désavantage marqué pour ce genre d'entreprise. Quant à l'analyse chimique seule, elle n'indique en rien ce qui peut être biodisponible pour les algues.

Conscients du fait qu'en gestion environnementale il importe tout d'abord de savoir s'il y a danger, la méthode dont nous envisageons le mise au point a pour but principal de démontrer le présence ou l'absence d'un potentiel de bioaccumulation d'un quelconque prélèvement liquide en exposant celui-ci à l'algue-test, le contenu cellulaire de laquelle est ensuite évalué en fonction de sa toxicité par l'entremise du système Microtox.

Pour atteindre ces objectifs, nous utilisons une algue, représentative de l'un des premiers maillons de la chaîne alimentaire, qui répond fort bien aux critères du *biomonitoring* (Phillips, 1977). Cette algue a fait l'objet de recherches intensives particulièrement en Amérique du Nord dans le but de développer une bioanalyse fiable et reproductible (USEPA, 1979); elle suscite un intérêt et une utilisation marqués dans les milieux scientifiques québécois (Blaise, 1980; Couture et collègues, 1981; Van Coillie et collègues, 1981; Joubert, 1980; Keighan, 1977), et sa polyvalence d'emploi en matière d'application environnementale est indiscutablement reconnu (Coutre, 1981). La communication présente fait état des travaux préliminaires réalisés dans le cadre de notre étude sur la bioaccumulation.

MATERIEL ET METHODES

L'essai consiste à exposer une population d'algues en phase de croissance logarithmique (période d'assimilation maximale) dans l'échantillon liquide pour une durée déterminée à l'avance. Les algues sont ensuite recueillies, brisées, et un test Microtox est effectué sur le lysat (contenu cellulaire). Parallèlement, on réalise un autre test Microtox sur le prélèvement étudié et son potentiel de bioaccumulation est alors évalué en considérant les résultats des deux tests.

Le choix des eaux évaluées fut fonction des échantillons dont disposaient nos laboratoires, alors que furent testés tantôt des prélèvements industriels ayant servi à une évaluation toxique par l'entremise du bio-essai avec la truite arc-en-ciel et tantôt des eaux de lixiviation préparées à partir de sédiments ou boues industrielles en utilisant la méthode suggérée par le Centre de Technologie des Eaux Résiduaires d'Environnement Canada (WTC, 1981). Dans ce dernier cas, les eaux de lessivage furent formées à partir d'une proportion de fraction liquide à fraction solide de 20:1. Comme traitement physique précédant la bioaccumulation, chaque échantillon fut soumis à une filtration sur membrane de 0.45 μ (Gelman GN-6). Un ajustement de pH fut fait lorsque celui-ci n'était pas entre 5 et 9.

Les principales conditions d'incubation des algues dans leur milieu synthétique se rapprochent de celles de la USEPA (USEPA, 1979):

inoculum d'algues	1000 cellules/mℓ
photopériode	24 heures (5,380 lux)
température	24 ± 2°C
agitation manuelle	2 fois par jour

Alors que dans de telles conditions, la population d'algues atteint la phase stationnaire en 8 jours, nous avions décidé, pour ces premiers essais, d'introduire l'eau des essais durant la phase exponentielle (jour 5) de croissance des algues en milieu synthétique 2x (deux fois concentré) pour une durée de contact de 24 ± 2 heures. Afin de travailler avec une biomasse d'algues raisonnable et pour augmenter la capacité du test Microtox à dépister un effet toxique bioaccumulateur, nous avons invariablement ajouté 200 mℓ d'échantillon à 200 mℓ de la culture d'algues du Jour 5, l'incubation se poursuivant dans un Erlenmeyer de 2 litres jusqu'au Jour 6. De façon identique, la culture d'algues de l'Erlenmeyer témoin recevait 200 mℓ d'eau Super Q (Millipore Super Q Water System) au Jour 5.

La mesure des populations d'algues au Jour 5 et au Jour 6 fut faite au moyen d'un compteur de particules (Coulter Counter, Modèle TA-11, orifice de 70 μ) et la transformation des comptes en biomasse fut ensuite établie grâce à la constante de production d'algues du milieu synthétique témoin.

Au Jour 6, la culture d'algues de chaque Erlenmeyer fut recueillie par centrifugation dans une série de huit éprouvettes coniques en verre d'une capacité de 50 mℓ chacune. Les huit culots furent combinés en un seul, qui fut lavé avec du $NaHCO_3$ (15 mg/ℓ) et soumis à deux autres rondes successives de centrifugation (2,000 RPM; 15 mn). Resuspendu à 2 mℓ avec du $NaHCO_3$, chaque culot d'algues passe ensuite à l'épreuve de l'ultrason (Branson sonifier, Model 350) où la rupture cellulaire s'effectue. On obtient alors le lysat de chaque culot en filtrant (Gelman GN-6; 0.45 µ) afin d'en séparer les parois brisées. Reconstitué ensuite à 10 ml avec de l'eau Super Q (Millipore Super Q Water System), le lysat fut salinisé (2%) et soumis à la bioanalyse par Microtox (Modèle 2055, Toxicity Analyzer System; Beckman Instruments, Inc.) selon la méthode indiquée dans le manuel d'instruction de l'appareil (Operating Instructions, 1980). La bioluminescence du réactif bactérien fut donc éprouvée en fonction de la toxicité présente dans chaque lysat. Afin d'exprimer la toxicité, le système Microtox utilise la notion suivante: CI_{50} (5 mn, $15^{o}C$), où:

- CI_{50} dénote la concentration d'une substance toxique ou d'une eau résiduaire quelconque occasionnant une réduction de 50% de l'intensité lumineuse normalement produite par le réactif bactérien;
- (5 mn, $15^{o}C$) dénote la durée totale de la bioanalyse où l'échantillon testé est en étroit contact avec le réactif bactérien à une température rigoureusement contrôlée de $15^{o}C$.

Il est parfois plus représentatif ou utile d'exprimer les résultats en unités toxiques (UT) selon le rapport suivant:

$$UT = \frac{100\%}{CI_{50}}$$

Cette formule indique que la toxicité, dans le cas d'un effluent par exemple, est inversement proportionnelle à la valeur de la CI_{50} en % v/v. Ainsi, plus la valeur des unités toxiques est élevée, plus toxique est l'effluent.

RESULTATS, DISCUSSION

Parmi les premiers efforts déployés pour mettre sur pied une méthode d'analyse de la bioaccumulation, il nous apparut important de réduire et même d'éliminer, dans la mesure du possible, la toxicité "parasite" du lysat témoin révélée par le Microtox, celle-ci variant généralement entre 1 et 10 unités toxiques (UT). Dans ce but, les teneurs maximales en oligo-éléments théoriquement bioaccumulables par les algues dans le milieu synthétique normal (USEPA, 1979) furent évaluées. Une solution de chacune des ces teneurs ainsi qu'un cocktail complet de celles-ci furent ensuite soumis au réactif bactérien du système Microtox. Ces résultats, présentés au tableau 1, démontrent un apport toxique du zinc, cobalt, cuivre, fer, ainsi que du cocktail lui-même. Une recette modifiée du milieu synthétique fut donc repensée afin de tenter d'éliminer tout "bruit" toxique vis-à-vis du Microtox. Au premier abord, il semble que ce milieu modifié de croissance utilisé pour les tests de bioaccumulation (MSB) n'altère nullement la production finale de biomasse d'algues en comparaison avec le milieu normal (tableau 2).

Toutefois, aucune diminution de toxicité significative ne fut notée au cours de tests Microtox subséquents réalisés sur des lysats d'algues cultivées dans le MSB. Les résultats démontrent que l'effet toxique est fonction de la biomasse d'algues reflétée par le lysat et que cette toxicité, vraisemblablement

Tableau 1. Modification de certaines teneurs en oligo-elements du milieu synthetique en fonction d'une toxicite au microtox

Element	Teneur normale (µg/ℓ)	Microtox (% d'inhibition)	Teneur modifiee (µg/ℓ)
B	32.46	Nul	-
Mn	115.374	Nul	-
Zn	15.691	14.4	0.157[1]
Co	0.354	11.8	0.07[2]
Cu	0.004	11.3	0.002[3]
Mo	2.878	Nul	-
Fe	33.051	15.5	55.0[4]
Cocktail	-	15.0	-

1. 1/100 : selon considérations émises par Chiaudani et Vighi 1978 et Greene et collègues 1975.
2. 1/5.06 : selon Chiaudani et Vighi 1978.
3. 1/2 : selon toxicité au Microtox.
4. 1.7 : $Fe\,Cl_3 \cdot 6\,H_2O$ remplacé par $Fe\,SO_4 \cdot 7\,H_2O$ (Chiaudani et Vighi 1978).

Tableau 2. Comptes d'algues[1] par mℓ obtenus apres 8 jours d'incubation en milieu synthetique normal (MSN) et en milieu synthetique modifie pour la bioaccumulation (MSB)

Milieu	Comptes (X10^6)	E.C.[2](X10^6)	C.V.[3]
MSN	5.42	0.59	10.9
MSB	5.41	0.35	6.5

[1] Tests réalisés avec 40 mℓ de milieu synthétique respectif dans 10 Erlenmeyers de 125 mℓ (biomasse théorique = 80 ppm) pour chaque milieu.

[2] E.C. (Ecart type).

[3] C.V. (coefficient de variation).

d'origine organique, semble liée aux composantes métaboliques de l'algue elle-même (figure 1). Conscients de ce bruit de fond, nous avons néanmoins testé une série d'eaux résiduaires au moyen de cette méthode d'analyse de la bioaccumulation. Le toxicité parasite de chaque lysat a tout simplement été retranchée en fonction de la biomasse selon la courbe (droite) de la figure 1, sans toutefois tenir compte des effets synergiques ou antagonistes qui auraient pu se manifester.

Nous avons utilisé la formule suivante pour le calcul de facteur de bioaccumulation (FB):

$$FB = \frac{UT_{LE} - UT_{LC}}{UT_{EFF}}$$

où:

- UT_{LE} représente les unités toxiques du lysat-effluent
- UT_{LC} représente les unités toxiques du lysat-contrôle
- UT_{EFF} représente les unités toxiques de l'effluent à la concentration testée (50%).

Les facteurs de bioaccumulation (FB) obtenus pour les différents échantillons testés apparaissent au tableau 3. Quatre classes de bioaccumulation arbitrairement établies en fonction des résultats obtenus montrent des FB variant de <1 à 30. Il est à noter que ces FB ne possèdent qu'une valeur relative de comparaison d'un effluent avec un autre, étant donné que nous n'avons pas tenu compte, pour ces essais préliminaires, de certains facteurs qui permettraient la possibilité de comparer. Ainsi, des travaux ultérieurs, que nous espérons réaliser à l'avenir, tâcheront de rendre directement comparables les FB obtenus pour divers rejets liquides. L'orientation des efforts futurs visera à éclairer les points suivants:

1. L'utilité de travailler avec une biomasse d'algues fixe et dont le lysat est non-toxique au réactif Microtox;
2. L'utilité de faire bioaccumuler les algues à partir d'une concentration-test d'eaux résiduaires correspondant à une valeur fixe d'unités toxiques;
3. L'utilité de bioaccumuler en utilisant des concentrations d'effluents sublétales pour les algues;
4. La possibilité de réduction de la durée du contact algues-effluent sans toutefois diminuer le potentiel de bioaccumulation;
5. La signification réele du potentiel de bioaccumulation mesuré au moyen du système Microtox.

Malgré ces problèmes qu'il faudra résoudre, il apparaît néanmoins probable qu'un heureux mariage, par l'utilisation de cette double bioanalyse algues-bactéries, puisse se matérialiser et contribuer aux études sur la bioaccumulation. Nous pensons, trop ambitieusement peut-être, qu'il serait possible de raffiner ce test au point où celui-ci serait en mesure de différencier entre

FIGURE 1 UNITES TOXIQUES DU CONTROLE EN FONCTION DE LA BIOMASSE ALGALE

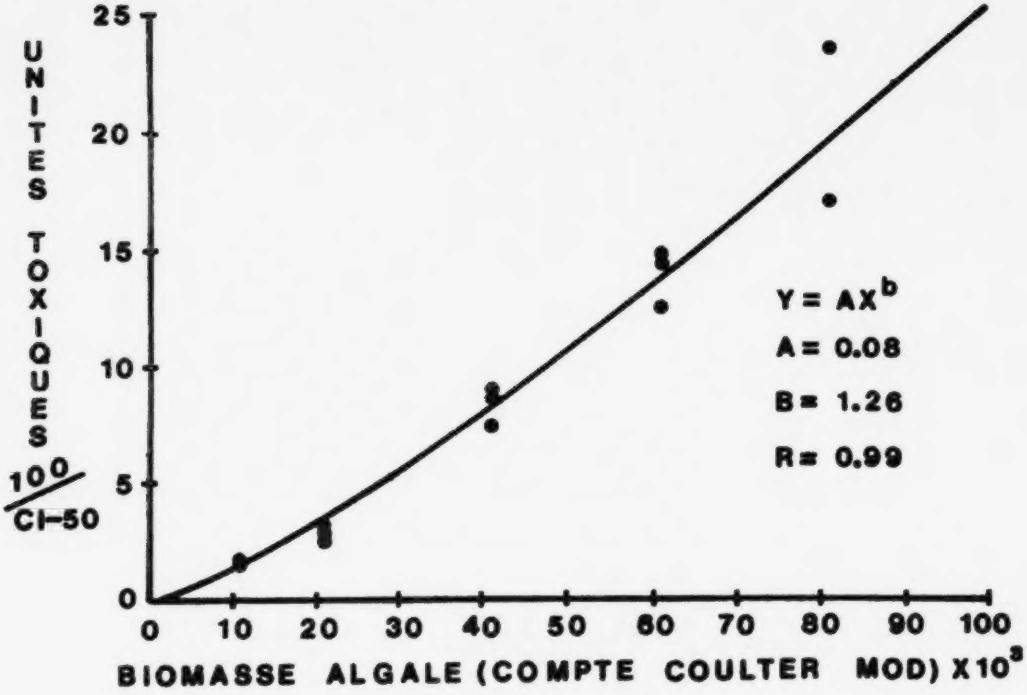


Tableau 3. Facteurs de bioaccumulation (FB) obtenus pour les echantillons testes

FB	Nombre de tests	Origine des echantillons[1]	Signification probable
< 1	31	LIX-BIP (12) LIX-SL (4) E-TM (2) E-PP (6) E-IP (7)	Aucun potentiel bioacc. (PB) et/ou antagonisme
1-3.9	5	LIX-BIP (1) E-PP (2) E-IP (2)	PB absent ou faible
4-9.9	4	LIX-BIP (1) E-IP (3)	PB moyen
10-30	4	E-R (1) E-SBA* (3)	PB fort
TOTAL	44	-	-

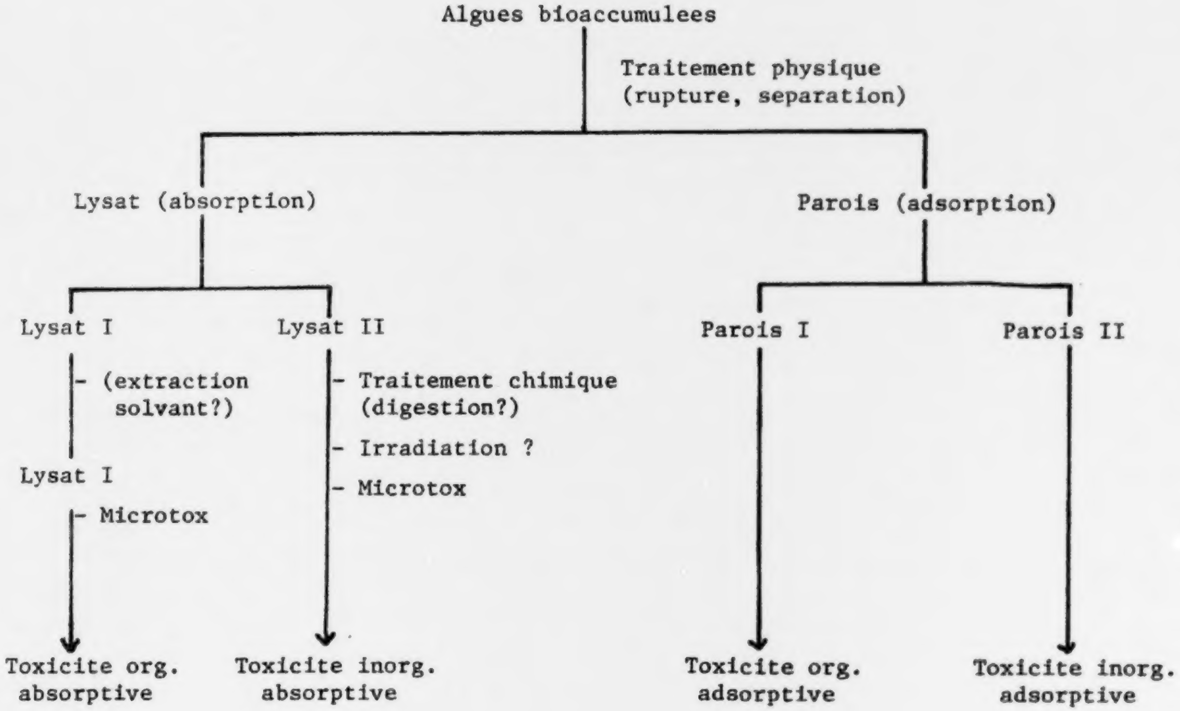
[1] LIX = lixiviation; BIP = Boues industrie pétrochimique; SL = sédiment lacustre.

E = effluent; TM = transformation de métal; PP = pâtes et pappier; IP = industrie pétrochimique; R = récupération (fondoir); SBA = sable bitumineux de l'Alberta.

( ) = nombre d'échantillons.

*FB maximum obtenu de 26.9.

Tableau 4. Raffinement possible du test de bioaccumulation


Algues bioaccumulees
Traitement physique
(rupture, separation)
Lysat (absorption)
Parois (adsorption)
Lysat I
Lysat II
Parois I
Parois II
- (extraction solvant?)
Lysat I
- Microtox
- Traitement chimique (digestion?)
- Irradiation ?
- Microtox
Toxicite org. absorptive
Toxicite inorg. absorptive
Toxicite org. adsorptive
Toxicite inorg. adsorptive

quatre types possibles de bioaccumulation chez les algues (tableau 4), soit un portentiel de bioaccumulation (PB) indicatif d'absorption ou d'adsorption minérale et/ou organique respectivement. Les applications possibles d'une telle methode d'analyse de la bioaccumulation seraient des plus intéressantes et pourraient rendre de précieux services, par exemple, dans le cadre d'études suivantes:

- études d'impacts sur les cours d'eaux;
- évaluation de systèmes de traitabilité;
- intégration dans un système étapiste d'évaluation de risques;
- études sur la spéciation et la biodisponibilité;
- élaboration de mesures de contrôle.

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THE EFFECT OF ETHANOL ON FUNGICIDE AND HERBICIDE TOXICITY ASSAYS WITH SELECTED TERRESTRIAL AND AQUATIC MICROORGANISMS

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BURRELL, R.E., and C.T. CORKE. 1981. The effect of ethanol on fungicide and herbicide toxicity assays with selected terrestrial and aquatic microorganisms. Can. Tech. Rep. Fish. Aquat. Sci.

The toxicity of ethanol to four fungi and a green alga was determined and the value of ethanol as a carrier solvent in fungal and algal bioassays was examined. When the fungicides benomyl and captan were introduced into a water-based medium with ethanol, at solvent concentrations of 1.0% (v/v) or greater, additive, antagonistic and synergistic interactions occurred depending upon the fungus involved. In herbicide assays, using linuron and atrazine, additive and antagonistic interactions were noted which varied with the concentration of ethanol.

It was concluded that ethanol is moderately fungitoxic and quite capable of interacting with fungicides and herbicides in bioassays. It is recommended that when ethanol is used as a carrier solvent the final concentration be kept to a minimum to avoid detrimental solvent-pesticide interactions.

Key words: ethanol, toxicity, interactions, fungi, alga.

BURRELL, R.E., and C.T. CORKE. 1981. The effect of ethanol on fungicide and herbicide toxicity assays with selected terrestrial and aquatic microorganisms. Can. Tech. Rep. Fish. Aquat. Sci.

L'étude ci-dessous a servi à déterminer la toxicité de l'éthanol vis-à-vis quatre champignons et une algue verte et à examiner l'utilité de l'éthanol en tant que solvant-porteur dans des essais biologigues sur les champignons et les algues. Lorsque les fongicides bénomyl et captan ont été ajoutés à un milieu à base d'eau contenant de l'éthanol d'une concentration de 1 p. cent (v/v) ou plus, des intéractions cumulatives, antagonistes et synergétiques se sont produites selon le champignon utilisé. Dans les essais à base d'herbicides, où on a utilisé du linuron et de l'atrazine, on a noté des interactions cumulatives et antagonistes dont l'intensité varie selon la concentration de l'éthanol.

Nous avons conclu que l'éthanol était modérément fongitoxique et tout à fait susceptible de réagir avec des fongicides et des herbicides dans des essais biologigues. Nous recommandons que lorsque l'éthanol est utilisé en tant que solvant-porteur, la concentration finale soit minimum afin d'éviter des interactions solvant-pesticides défavorables.

INTRODUCTION

Organic solvents have been used to introduce organic pesticides into water-based biological test systems (Manten et al. 1950). Until recently, there has been very little evaluation of organic solvents to determine their suitability for this purpose. Some reports suggested that solvent levels as high as 2.0% (v/v) had no effect on test fungi (Manten et al. 1950). Conclusions such as this eventually led to the accepted use of 1.0% acetone (Edgington et al. 1971) and 1.0% ethanol (Nishimura et al. 1973) as carrier solvents in bioassays. In fact, so little importance was placed on the carrier solvent used in bioassays that some researchers neglected to mention them in their publications (Buchenauer and Erwin 1976; Ali et al. 1979). However, there are reports which indicate that selection of carrier solvent may be a very important consideration when planning bioassays of water-insoluble pesticides or industrial pollutants. Ghendon and Samoilova (1960) reported that 1.0% acetone reduced vaccinia and rabbitpox virus replication by 90-95%. Parasher et al. (1978) found that the growth and ultrastructure of the green alga *Chlorella pyrenoidosa* were affected by high concentrations of acetone (3.33% v/v) but only growth was affected at low concentrations (0.33% v/v). Dalela et al. (1979) found that chlordane, used in conjunction with different solvents, resulted in various levels of ATPase inhibition in the gills and brains of freshwater teleosts. Bowman et al. (1981) reported that the toxicity of some pesticides can be greatly increased by methanol and dimethyl sulfoxide. Burrell et al. (1980) and Burrell and Corke (1980) have reported on the theory of interactions that may occur in bioassays with fungi and blue-green algae when acetone was the carrier solvent.

In this paper, the effects of ethanol on the growth of several fungi and a green alga are detailed. The effects of ethanol on fungicide and herbicide bioassays are reported.

MATERIALS AND METHODS

CULTURES

Pure cultures of *Fusarium oxysporum* f. sp. *lycopersici*, *Pestalotia* sp., *Polyporus hirsutus* and *Sclerotinia homeocarpa* were supplied by the Department of Environmental Biology, University of Guelph. They were maintained on Potato Dextrose Agar (Difco) at 22°C. An axenic culture of the green alga, *Chlorella pyrenoidosa*, which was maintained in a liquid nitrogen-supplemented medium at 22°C and 7000 lux with a 16 h light: 8 h dark photoperiod (Stratton and Corke 1979).

FUNGAL ASSAYS

Ethanol was added to 100 mL bottles of molten Potato Dextrose Agar (PDA) to give final concentrations of 0 (control), 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0% (v/v). The bottles of agar were shaken for two minutes on a rotary shaker and then 10 mL volumes were dispensed into plastic Petri dishes and allowed to solidify. A mycelial disc, 8 mm in diameter, taken from the outer edge of a 6-day-old culture was placed at the centre of each Petri dish. The inoculated Petri dishes (replicates of 5) were incubated at 30°C until control colony diameters exceeded 40 mm (48 - 72 h). All colony diameters were then measured. Percent inhibitions of growth were calculated and plotted on logarithmic-linear graph paper as log concentration of ethanol versus percent inhibition.

Ethanol-fungicide interactions were determined using the method of Burrell and Corke (1980). Single concentrations of benomyl [methyl-1-(butylcarbamoyl) benzimidazol-2-ylcarbamate] and captan [N-(trichloromethylthio)-cyclohex-4-ene-1,2-dicarboximide] were used with ethanol concentrations that were varied from 0.1 to 2.0% (v/v). The concentrations of benomyl utilized were 0.1 µg/mL with *S. homeocarpa* and *Pestalotia* sp. and 1.0 µg/mL with *F. oxysporum* and *P. hirsutus*. Captan was used at 10 µg/mL with all cultures. Solvent controls were prepared as above and solvent-fungicide combinations, using the same levels of ethanol, were prepared with the appropriate concentration of fungicide. The growth from these treatments was compared to that on the absolute controls (no solvent and no pesticide) to determine solvent effects and solvent-fungicide effects. Percent inhibitions were calculated also, with respect to solvent controls, from the solvent-fungicide treated growth plates to yield a net-fungicide effect. Percent inhibition data were plotted on logarithmic-linear graph paper.

Using concentrations of 0.1 and 1.0% (v/v) ethanol, the fungitoxicities of captan and benomyl were determined. Percent inhibitions were calculated from the appropriate solvent controls and data were plotted on logarithmic-linear graph paper. EC_{50} values were obtained from these graphs.

ALGAL ASSAYS

The effects of various ethanol concentrations, 0 (control), 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5 and 2.0% (v/v) on the growth of *Chlorella pyrenoidosa* were determined in liquid nitrogen-supplemented medium. The medium was dispensed into test tubes and the appropriate volumes of ethanol were added to give the desired final concentrations and a total volume of 10 mL. The test tubes were inoculated with 10^6 cells (final cell count was 10^5 cells/mL) and incubated at 22°C and 7000 lux with a photoperiod of 16 h light: 8 h dark. The growth in each test tube was followed optically (λ420 nm) on a Spectronic 20 for 12 days. The data were plotted on linear graph paper as optical density versus time in days.

Solvent-herbicide interactions were determined for the herbicides linuron [3-(p-chlorophenyl)-1-methoxy-1-methylurea) and atrazine [2-chloro-4-(ethyl-amino)-6-(isopropylamino)-s-triazine] with 0.1 and 1.0% (v/v) ethanol. Test tubes, containing 10 mL of liquid nitrogen-supplemented medium, 0.1% or 1.0% (v/v) ethanol and either linuron or atrazine, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 µg/mL, were inoculated and incubated as above. Growth was followed optically (λ420) and these data were plotted on linear graph paper as optical density versus time (days). Percent inhibition of growth, at day 10, was calculated for each herbicide concentration from the appropriate ethanol control. These calculations yielded ethanol corrected and uncorrected toxicity data which were plotted on logarithmic-linear graph paper as percent inhibition versus log herbicide concentration.

STATISTICS

Statistical conclusions were made using a Dunnett's test for comparison of means at $\alpha = 0.02$ (Winer 1971).

RESULTS AND DISCUSSION

FUNGAL ASSAYS

The toxic effects of ethanol are summarized in Table 1. The most sensitive organism was *Sclerotinia homeocarpa*, an Ascomycotina, with an EC_{50} of 1.0% ethanol (v/v) while the least sensitive organism was *Polyporus hirsutus*, a Basidiomycotina, for which an EC_{50} could not be derived as the maximum concentration of ethanol used (2.0% v/v) resulted in only a 42% inhibition of growth. The two Deuteromycotina, *Pestalotia* sp. and *F. oxysporum*, had EC_{50} values of 1.15 and 1.9% (v/v), respectively. The toxicity curves for all 4 fungi were very similar as each curve had a point of inflection that fell between 0.7 and 0.9% (v/v) ethanol Table 1). This similarity in curve appearance was noted also in previous publications dealing with the effects of acetone on fungi (Burrell and Corke 1980).

Table 1. Effect of ethanol on fungal growth

Fungus	% Inhibition of growth due to four ethanol concentrations (% v/v) 0.1	0.5	1.0	2.0	Point inflection (% Ethanol)	EC_{50} of ethanol (% v/v)
Polyporus hirsutus	0	7	14	42	0.9	> 2.0
Pestalotia sp.	8	21	40	95	0.7	1.15
Sclerotinia homeocarpa	8	22	53	98	0.7	1.0
Fusarium oxysporum	0	8	15	52	0.8	1.9

Previous reports have outlined the theoretical interactions that may occur between solvents and pesticides in bioassays (Burrell and Corke 1980; Burrell et al. 1980). All three types of theoretical interactions, additive, antagonistic and synergistic, were observed when ethanol was used as the carrier solvent for benomyl and captan. The ethanol-fungicide interactions as determined by the graphical technique of Burrell and Corke (1980) are outlined in Table 2. Toxicity curves generated, using 0.1 and 1.0% ethanol (v/v) as the carrier solvent, were used to calculate EC_{50} values for verification of the interactions. These EC_{50} values are listed in Table 3 with the types of interactions they indicated. There was complete agreement between the two methods.

The similarities between ethanol toxicity curves and those representing acetone suggest that the modes of action of the solvents are similar. If that were true, then the interactions of the solvents with the fungicides and fungi should be similar. To verify this, a comparison of the interaction data presented here was made with those data which were previously published (Table 4). Three of the fungi, *Pestalotia* sp., *P. hirsutus* and *S. homeocarpa* responded similarly regardless of the solvent used. *Fusarium oxysporum* produced different results. Acetone and captan were synergistic (Burrell and Corke 1980) while ethanol and captan were clearly antagonistic. Similar differences were observed with the fungicide benomyl. These differences indicate that although the solvents may be similar in their modes of action, there are subtle differences between them. Clearly data for one solvent, cannot be applied to another; each solvent must be analyzed independently for its interactive properties.

Table 2. Ethanol-fungicide interaction

Fungus	Fungicide (μg/mL)	% Inhibition of growth caused by benomyl and captan at two ethanol concentrations (% v/v) 0.1	2.0	Response
P. hirsutus	Captan (10 ppm)	53	55	Additive
	Benomyl (1.0 ppm)	7	8	Additive
Pestalotia sp.	Captan (10 ppm)	76	24	Antagonism
	Benomyl (0.1 ppm)	10	22	Synergism
S. homeocarpa	Captan (10 ppm)	41	100	Synergism
	Benomyl (0.1 ppm)	18	32	Synergism
F. oxysporum	Captan (10 ppm)	49	30	Antagonism
	Benomyl (1.0 ppm)	50	51	Additive

Table 3. EC_{50} values determined for captan and benomyl at 0.1 and 1.0% ethanol with four fungi

Fungus tested	Fungicide	EC_{50} (ppm) at two ethanol concentrations (% v/v) 0.1	1.0	Response
P. hirsutus	Captan	9.5 ± 1.0	7.5 ±1.0	Additive
	Benomyl	2.8 ± 0.04	3.2 ±0.3	Additive
Pestalotia sp.	Captan	9.4 ± 0.3	>30[1]	Antagonistic
	Benomyl	0.12 ± 0.001	0.095±0.007[1]	Synergistic
S. homeocarpa	Captan	11.5 ± 0.3	5.0 ±0.2[1]	Synergistic
	Benomyl	0.32 ± 0.008	0.22 ±0.01[1]	Synergistic
F. oxysporum	Captan	12.0 ± 1.0	30.0 ±1.1[1]	Antagonistic
	Benomyl	1.0 ± 0.05	0.95 ±0.004	Synergistic

[1] Significantly different EC_{50}s at $P = 0.02$

Table 4. A summary of the solvent-fungicide interactions found using benomyl and captan with acetone[1] and ethanol

	Solvent and Fungicide			
Fungus	Acetone & captan	Ethanol & captan	Acetone & benomyl	Ethanol & benomyl
F. oxysporum	Synergism	Antagonism	Synergism	Additive
Pestalotia sp.	Antagonism	Antagonism	Synergism	Synergism
P. hirsutus	Additive	Additive	Additive	Additive
S. homeocarpa	Synergism	Synergism	Synergism	Synergism

[1] Acetone data from Burrell & Corke (1980)

ALGAL ASSAYS

Ethanol was moderatly toxic to the growth of the green alga *C. pyrenoidosa* (Fig. 1). As the concentration of ethanol was increased there was a marked drop in growth of the alga. The calculated EC_{50} value for ethanol and this organism was 0.35% (v/v).

The toxicity response by *C. pyrenoidosa* to the herbicide linuron was determined at two different levels of ethanol, 0.1 and 1.0% (v/v). The ethanol-corrected percent inhibitions determined with 0.1% ethanol (54%) and 1.0% ethanol (24%) for 0.5 μg/mL linuron differed by 30% (Fig. 2). This response clearly showed that 1.0% ethanol antagonized the linuron. When the toxic response to atrazine was examined at two different ethanol levels, a similar phenomenon was observed (Fig. 3). There was a 16% difference in ethanol-corrected toxicities for the two concentrations of ethanol used.

SUMMARY

The data presented here show that ethanol can interact in a variety of ways with pesticides in bioassays. It is important that interactions such as these be minimized through experimental design. When solvents must be used at concentrations of 1.0% (v/v) or higher, considerable experimentation must be undertaken to separate synergistic and antagonistic responses from those attributed to the actual pesticide or pollutants.

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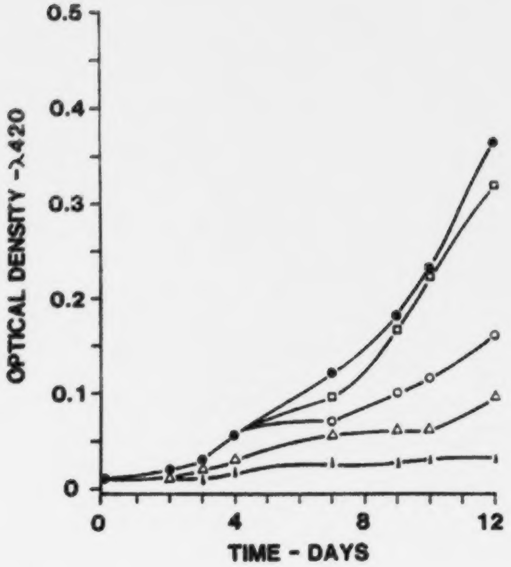

FIG. 1. Growth of *Chlorella pyrenoidosa* in the presence of varying concentrations of ethanol.

(●) - control; (O) - 0.4%; (Δ) - 0.8%; (Λ) - 2.0%; (□) - 0.1%.

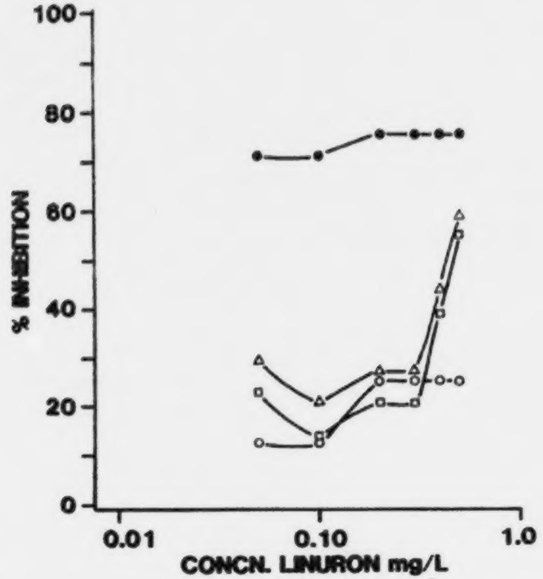

FIG. 2. Percent inhibition of the growth of *Chlorella pyrenoidosa* caused by linuron and ethanol.

(●) - 1.0% ethanol--uncorrected; (O) - 1.0% ethanol--corrected
(Δ) - 0.1% ethanol--uncorrected; (□) - 0.1% ethanol--corrected

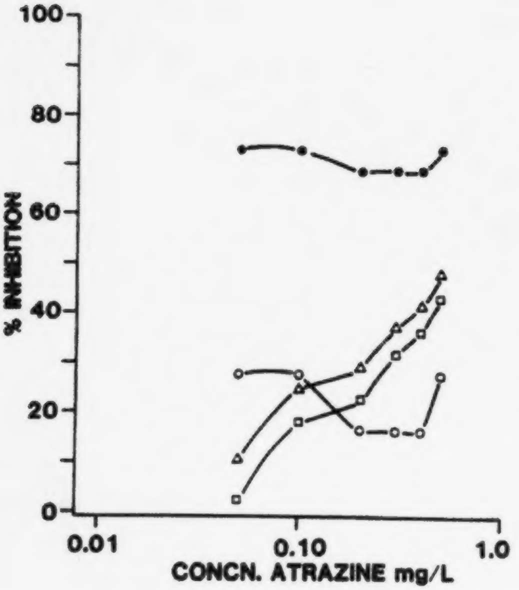

FIG. 3. Percent inhibition of the growth of *Chlorella pyrenoidosa* caused by atrazine and ethanol.

(●) - 1.0% ethanol--uncorrected; (0) - 1.0% ethanol--corrected
(Δ) - 0.1% ethanol--uncorrected; (□) - 0.1% ethanol--corrected

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THE EFFECTS OF LOW pH, SELENIUM AND CALCIUM ON THE BIOACCUMULATION OF ^{203}Hg BY SEVEN TISSUES OF THE CRAYFISH, *ORCONECTES VIRILIS*

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The effects of low pH (5.4 and 5.0), selenium (10 µg L^{-1}) and elevated calcium concentrations (5 and 15 mg L^{-1}) on the bioaccumulation of radioactive isotopes of Hg, Se, Cd and Zn by seven tissues of the crayfish, *Orconectes virilis*, were studied in 1 m diameter enclosures in Lake 302, Experimental Lakes Area, northwestern Ontario. Tubes contained lake communities plus added pearl dace, white suckers and crayfish. Only bioaccumulation of ^{203}Hg by the crayfish is discussed here.

Concentration of ^{203}Hg increased with time in seven tissues of the crayfish until at least 15 days after isotope addition. General rank order of ^{203}Hg accumulation by tissues (cpm g^{-1} wet weight) was green glands>hepatopancreas>gills>gut>carapace>ovary>abdominal muscle. Low pH in a number of cases appeared to retard accumulation of ^{203}Hg in tissues, selenium consistently retarded bioaccumulation, and elevated calcium concentrations were associated with a few cases of enhanced ^{203}Hg bioaccumulation. ^{203}Hg declined exponentially in the water of tubes. Loss rate of ^{203}Hg was significantly faster in the tubes with selenium and was associated with a higher proportion of the ^{203}Hg bound to suspended particulates than in other tubes. Water in tubes contained spuriously high concentrations of zinc, 30 to 200 µg L^{-1} compared with lake water of <10 µg L^{-1}. Effect of high zinc concentration on bioaccumulation of ^{203}Hg by crayfish is unknown.

Key words: low pH; selenium; mercury; calcium; crayfish tissues; bioaccumulation.

CHANG, P.S.S., D.F. MALLEY, N.E. STRANGE and J.F. KLAVERKAMP. 1981. The effects of low pH, selenium and calcium on the bioaccumulation of ^{203}Hg by seven tissues of the crayfish, *Orconectes virilis*. Can. Tech. Rep. Fish. Aquat. Sci.

Les effets d'un pH faible (5.4 et 5.0), du sélénium (10 µg ℓ^{-1}) et d'une concentration élevée de calcium (5 et 15 mg ℓ^{-1}) sur la bioaccumulation de quatre isotopes radioactifs de Hg, Se, Cd et Zn par sept tissus d'écrevisse ont été étudié dans des enclos de 1 m de diamètre au lac 302 de la Région de Lacs Expérimentaux du nord ouest ontarien. Chaque tube contenait un échantillon représentatif de la communauté aquatique de lac et on y avait ajouté des mulets perlés, des meuniers noirs et des écrevisses. Seule la bioaccumulation de ^{203}Hg par l'écrevisse est discutée.

Les concentrations de ^{203}Hg augmentent progressivement dans sept tissus d'écrevisse pendant au moins 15 jours après son introduction. La concentration de ^{203}Hg dans les différents tissus (cpm g^{-1} poids frais) est de l'ordre suivant: glandes vertes hépatopancréas>branchies>intestin>carapace>ovaire>muscle abdominal. Un faible pH semble inhiber dans plusiers cas l'accumulation de ^{203}Hg par les tissus, le sélénium retarde de façon consistante la bioaccumulation et les concentrations de calcium élevées sont liées dans quelques cas à une accumulation accrue de ^{203}Hg. Le ^{203}Hg diminue exponentiellement dans l'eau des tubes. Le taux de disparition est significativement plus rapide dans les tubes contenant du sélénium et y est associé à une proportion plus élevée de ^{203}Hg lié aux particules en suspension que dans les autres tubes. L'eau a l'intérieur des tubes contient des concentrations anormalement élevées de zinc, 30 à 200 µg ℓ^{-1}, comparees a celles du lac (<10 µg ℓ^{-1}). Les effets de la concentration élevée de zinc sur la bioaccumulation de ^{203}Hg par les tissus d'écrevisse ne sont pas connus.

INTRODUCTION

An experiment to study the effects of low pH and additions of selenium or calcium on the behaviour of the radioisotopes, ^{109}Cd, ^{75}Se, ^{203}Hg and ^{65}Zn, was conducted from mid-August to mid-October, 1980 in a series of 1 m diameter enclosures (tubes) in Lake 302 in the Experimental Lakes Area (ELA), northwestern Ontario. It was designed as an initial study to determine whether acidification, the addition of selenium or elevated calcium concentrations have major effects on the rates of accumulation of these isotopes by various biological and physical compartments in small aquatic ecosystems. These compartments included water, suspended particulates, zooplankton, chlorophyll, periphyton, crayfish, white suckers and pearl dace.

Low pH altered the solubility of radioisotopes of several heavy metals in and their rate of loss from the water column in 10 m diameter tubes in another ELA lake (Schindler et al. 1980a). The presence of selenium in 10 m diameter tubes in Clay Lake, northwestern Ontario, affected the movement of mercury among various compartments in the water column and retarded rate of mercury bioaccumulation by fish, crayfish and haptobenthos (Rudd et al. 1980). Calcium has been found to reduce bioaccumulation of zinc by the crayfish *Austropotamobius pallipes pallipes* (Bryan 1967) and cadmium by the amphipod *Gammarus pulex* (Wright 1980). Calcium concentration in lake water appeared to be inversely related to accumulation of several heavy metals in liver of two fish species (McFarlane and Franzin 1980).

This paper presents data on the uptake of ^{203}Hg by seven tissues of the crayfish, *Orconectes virilis*. Results on the bioaccumulation of the other three isotopes by the crayfish will be reported elsewhere. General experimental design and the behaviour of the four isotopes in other compartments will be presented by Klaverkamp et al. (in prep.).

MATERIALS AND METHODS

DESCRIPTION OF THE LAKE

Lake 302 is a double-basin lake located at 49°41'N latitude and 93°46'W longitude in the Experimental Lakes Area, northwestern Ontario (Brunskill and Schindler 1971). North and south basins have surface area and maximum depth of 12.8 ha, 13.8 m and 10.9 ha, 10.6m, respectively. The north basin of the lake has been used previously to study the effects of the injection of nutrients into the hypolimnion. Additional lake morphometry and the results of that experiment are described by Schindler et al. (1980b). Average composition of epilimnetic water of the south basin between June 10 and October 28, 1980 is given in Table 1.

TUBE CONSTRUCTION AND TREATMENTS

The experiment was conducted in fifteen 1 m diameter enclosures (tubes) in 2.5 to 3 m of water in a sheltered bay of the south basin of Lake 302. These tubes were constructed of cross-laminated polyethylene. The top openings of the tubes were supported by a wood and styrofoam frame. PVC tubing was inserted into the tubes to provide additional structural strength. Galvanized metal culverts, 1 m in diameter, were placed externally on the bottom skirts of the tubes to seal tubes to the sediments. The skirts were further stabilized on the sediments by sandbags.

The tubes were arranged in 5 series of 3 tubes each, each series consisting of tubes labelled A, B and C (Table 2). Sufficient NaCl was added to each tube on August 18 to increase Na^+ concentration about 3-fold above the lake background average of 0.69 mg L^{-1} (N = 6 between August 15 and September 16) in order to monitor tubes for leakage. No major leakages were detected during the experiments. Half-times for Na^+ concentration in tubes to return to background level ranged from 17 to more than 200 days, but most commonly were about 70 days (Table 3). Half-times were calculated from 6 water samples between 0 and 58 days, but the last data point was dropped when this was lower than the trend between 0 and 29 days.

Beginning on August 18, B and C tubes in all series were acidified to average pH 5.44 and 5.01, respectively, using 0.1 N H_2SO_4. The pH of these tubes was held constant by the addition of acid on a daily basis. Tubes A were allowed to remain at average lake pH of about 6.73.

On August 15, prior to acidification, Na_2SeO_3 was added to tubes in series 2. Concentrations declined with time (Table 4) and were intended to be approximately 10 µg L^{-1} on the day of isotope addition. On August 18, $CaCl_2$ was added to series 3 and 4 to bring tubes to nominal concentrations of 5 mg L^{-1} and 15 mg L^{-1}, respectively. Ca^{++} in series 3 tubes remained very constant up to 22 days, after which it declined slowly in A and B but remained constant in C. In series 4 tubes Ca^{++} declined exponentially with time and half-times of disappearance of the added Ca^{++} ranged from 48 to 88 days (Table 5). Tubes in series 1 and 5 received no chemical additions other than acid.

On August 26 (designated day 0) each tube received 0.33 mCi of each of ^{203}Hg, ^{109}Cd, ^{65}Zn and 0.13 mCi of ^{75}Se (1 Ci = 37 GBq). A summary of the

Table 1. Average composition ($\bar{x}$ ±S.E.) of epilimnetic water sample between June 10 and October 28, 1980 from Lake 302.

No. of samples	Conductivity µS cm^{-1} at 25°C	Dissolved inorganic carbon, µmoles L^{-1}	pH range
6	23.0±1.7	73.8±8.7	6.40-6.74

Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	SO_4^{--}
mg L^{-1}					
0.85±0.04	0.38±0.02	1.59±0.02	0.58±0.01	2.03±0.08	2.98±0.07

Table 2. Treatments to tubes in Lake 302, 1980.

Tube series	Tube (mean pH in parentheses)			Additions
1	A(6.7)	B(5.4)	C(5.0)	isotopes[a] + H_2SO_4[b]
2	"	"	"	10 µg L^{-1} selenium + isotopes[a] + H_2SO_4[b]
3	"	"	"	5 mg L^{-1} calcium + isotopes[a] + H_2SO_4[b]
4	"	"	"	15 mg L^{-1} calcium + isotopes[a] + H_2SO_4[b]
5[c]	"	"	"	isotopes[a] + H_2SO_4[b]

[a] $^{109}Cd + ^{75}Se + ^{203}Hg + ^{65}Zn$

[b] Tubes B and C only.

[c] Replicate of series 1.

Table 3. Half-times for disappearance of added Na^+ from water in tubes in Lake 302. Half-times were calculated between 0 and 58 days except for values marked by * which denotes calculations over 0 to 29 days.

Series	Tube	Half-times of disappearance of added Na^+, days		
		A	B	C
1		46.0	78.6	58.9
2		19.9*	69.3	29.6*
3		17.1*	70.9	155.6
4		112.2	70.5	84.3
5		200+	181.3	200+

Table 4. Concentrations of Se in tubes of series 2 initially after its addition, at the time of addition of isotopes 11 days later, and half-time of disappearance.

Tube	Concentration of Se, μg L^{-1}		
	Initially after addition	On day of isotope addition	Half-times of disappearance, days
2A	21.6	7.6	11.3
2B	23.7	7.4	15.1
2C	33.4	5.8	7.7

Table 5. Concentrations of Ca^{++} in tubes of series 3 and 4 and half-times of disappearance of added Ca^{++}.

Tube	Initial Conc. mg L^{-1}	Average Conc. 0-22 d mg L^{-1}	Half-times of disappearance, days
3A	4.71	4.60 ± 0.01	-
3B	5.07	5.11 ± 0.04	-
3C	5.24	5.08 ± 0.06	-
4A	13.9	-	88.0
4B	14.3	-	48.1
4C	14.0	-	48.8

nominal treatments of each tube is given in Table 2. The introduction of fish, periphyton strips and water sampling scheme are described by Klaverkamp et al. (in prep.).

COLLECTION AND SAMPLING OF CRAYFISH AND ANALYSIS OF TISSUES

All crayfish were collected at night from another ELA lake, 239, at a depth of less than 1 m of water. Only mature females were selected for the experiment because they had completed a molt earlier in the summer and would not molt again. Crayfish were weighed and measured and placed singly into individual compartments (12 x 12 x 7 cm) of wire-mesh cages (60 x 12 x 7 cm). These cages were previously covered with several coats of Varathane® to ensure that no bare metal was exposed. Caged crayfish were maintained in Lake 239 in shallow water for several days before the experiment began.

The day before the addition of isotopes, caged crayfish were transported to Lake 302. Two cages containing a total of 10 crayfish were introduced into each of the 15 tubes and allowed to rest on the bottom. No food was added to the tubes during the experiment.

Two to four crayfish, normally three, were removed from each tube at 9 ± 2, 15 ± 1 and 35 ± 1 day, after isotope introduction. The radioactive crayfish were transported to the ELA laboratory where wet weights, carapace lengths and molt stages were recorded. Crayfish were dissected to obtain the following tissues for isotope analyses: carapace, hepatopancreas, gills, green glands (antennal glands), ovary, gut (proventriculus and intestine) and abdominal flexor muscle. Fresh tissue samples were placed in plastic petri dishes (50 mm in dia., 9 mm in height) with locking lids and were kept at 5°C. Length of time required for dissection determined the number of days required for sampling. Crayfish were dissected within a few hours of removal from a tube.

The activities of the four isotopes in the tissues were determined within 4 months of the isotope addition to the tubes by gamma spectroscopy performed on a lithium-drifted germanium (GeLi) crystal detector connected to a 4000 channel analyzer. Spectra were processed by a programmable computer (Hesslein et al. 1980). Samples were counted for an appropriate time period, usually 1 to 2 h. Samples were corrected for radioactive decay by normalizing the counts to the day of isotope addition to the tubes. Data were expressed as counts per minute per gram wet weight of tissue (cpm g^{-1}). Water and particulate samples were analyzed for the activity of the four isotopes according to Hesslein et al. (1980).

Ionic and other analyses on water were performed using the methods of Stainton et al. (1977). Concentrations of zinc in water samples and tissues of crayfish were determined by the Analytical Services Laboratory of the Freshwater Institute (A. Lutz, unpublished methods).

DATA ANALYSIS AND STATISTICAL TESTS

The disappearances of ^{203}Hg from the water in the tubes was modelled by:

$$C_t = C_o e^{-bt}$$

or its linear form:

$$\ln C_t = \ln C_o - bt$$

where C_t is cpm L^{-1} ^{203}Hg at time t, C_o is the cpm L^{-1} ^{203}Hg at time 0, b is the loss rate and t is time in days. The parameters C_o and b were estimated by linear regression. Half-time of disappearance was calculated as ln2/b. 95% confidence interval around each loss rate, b, was calculated from tabulated Student's *t* and the standard error of b (Steel and Torrie 1960). Confidence intervals around the half-times were calculated from the upper and lower limits of b.

In order to combine data from various tubes which differed somewhat in C_o, data from several tubes were expressed as ln C_t/C_o, fraction of the initial cpm L^{-1} of ^{203}Hg remaining at time t, and regressed against time. Loss rates from two sets of pooled data were compared using Student's *t*-test to determine homogeneity of regression coefficients (Steel and Torrie 1960).

For comparisons of content of ^{203}Hg in tissues from tubes differing somewhat in C_o, cpm g^{-1} of wet weight of tissue were expressed as bioconcentration factors:

$$\frac{\text{cpm g}^{-1}\text{ tissue at time } t}{\text{cpm mL}^{-1}\text{ water at time 0 } (= C_o/1000)}$$

Differences in average bioconcentration factors at different sampling times and from different experimental conditions were compared using Tukey's ω procedure (Steel and Torrie 1960).

RESULTS AND DISCUSSION

BEHAVIOUR OF ^{203}Hg IN THE WATER COLUMN

Total ^{203}Hg (cpm L^{-1}) in the water column declined exponentially over the period of 43 days (Table 6). Half-times of disappearance of this isotope from the water ranged from 6.6 to 14.3 days with an average of 10.8 days (Table 7). When all series were pooled, ^{203}Hg was removed at a faster rate from A tubes, at near neutral pH, than from acidified B and C tubes (Table 8). This is reflected in generally shorter half-times of disappearance for A tubes (Table 7). The rates of removal of ^{203}Hg in the series 2 tubes (with Se) were faster than in all other tubes combined (Table 8). Elevated calcium concentration (series 3 and 4) did not affect rate of disappearance of ^{203}Hg (Table 8)

Table 6. Total ^{203}Hg (cpm L^{-1}) in the water column one day after addition of isotopes to 1 m diameter tubes in Lake 302, 1980. Values under days 3 to 43 are the total ^{203}Hg in the water column expressed as a percentage of the day 1 value.

	Days after additon of isotopes					
Tube	1	3	7	14	22	43
		% of day 1				
1A	8986	85	91	40	26	5
1B	7932	91	92	84	45	13
1C	7718	88	91	44	25	8
2A	8163	80	82	49	6	2
2B	9487	78	87	59	27	7
2C	7166	81	92	39	15	3
3A	7342	83	78	37	6	4
3B	7282	81	74	40	24	8
3C	8378	86	77	50	26	13
4A	8413	76	88	34	32	8
4B	9210	89	90	34	31	7
4C	11325	78	84	39	35	7
5A	10474	74	80	28	21	6
5B	7986	83	93	27	30	9
5C	10531	75	63	36	42	7

Table 7. Half-times in days for disappearance of ^{203}Hg from the water column of tubes in Lake 302, 1980. 95% confidence intervals are given in parentheses.

Tube unit	1	2 (Se)	3 (Low Ca)	4 (High Ca)	5
A (pH 6.7)	9.8 (8.4-11.8)	6.6 (4.8-10.6)	8.2 (5.5-16.3)	11.9 (9.2-16.8)	10.4 (8.2-14.2)
B (pH 5.4)	14.3 (10.8-21.3)	10.7 (8.7-13.7)	11.7 (10.3-13.5)	10.6 (8.6-14.0)	12.0 (8.5-20.8)
C (pH 5.0)	10.9 (9.3-13.3)	8.0 (6.6-9.9)	13.9 (11.3-17.9)	11.5 (9.3-15.2)	12.0 (9.0-18.1)

Table 8. Statistical test (homogeneity of regression coefficients) for differences in rates of disappearance of total ^{203}Hg from the water columns of tubes in Lake 302, 1980.

Data pooled for regression analysis	Probability that the regression coefficients are not different
1A vs 1B, 1C	$P > 0.05$
2A vs 2B, 2C	$P < 0.001$
3A vs 3B, 3C	$P < 0.02$
4A vs 4B, 4C	$P > 0.1$
5A vs 5B, 5C	$P > 0.2$
all A tubes vs all B tubes	$P < 0.01$
all A tubes vs all C tubes	$P < 0.02$
series 2 vs series 1, 3, 4 and 5	$P < 0.001$
series 3 and 4 vs series 1 and 5	$P > 0.5$

Table 9. Average percentages of total ^{203}Hg associated with particulates (± S.E.). Data from the A, B and C tubes in each series were pooled. Lines connect pairs which are different at the 0.01 level using Tukey's ω procedure. All other pairs were not different at the 0.05 level.

1	2	3	4	5
39.7 ± 2.3	54.7 ± 3.4	43.7 ± 2.5	38.4 ± 2.8	40.0 ± 3.6

Table 10. Average percentages of total ^{203}Hg associated with particulates (± S.E.). Data from series 1 to 5 are pooled in each comparison. Lines connect pairs which are different at the 0.05 level using Tukey's ω procedure.

A pH 6.7	B pH 5.4	C pH 5.0	B + C
40.3 ± 2.5	46.5 ± 2.5	43.1 ± 2.5	44.8 ±

or half-time of disappearance (Table 7) compared with series 1 and 5 without added calcium.

Proportions of the total ^{203}Hg in the water column were associated with suspended particulates. Similarly to total ^{203}Hg, particulate ^{203}Hg declined exponentially with time in all tubes ($b = 0$, $P < 0.05$ for all tubes). Percentage of ^{203}Hg associated with particulates ranged from 17 to 80% and in 12 of the 15 tubes showed no tendency to change with time. Three tubes (1A, 4B, 4C) showed small increases in proportion of ^{203}Hg associated with particulates with time ($b = 0$, $P < 0.05$). The percentage of ^{203}Hg in the particulate material was significantly higher in the Se tubes (55%) than in those without the addition of Se (Table 9). There were no differences between other series (38-44%). Thus, elevated levels of calcium had no effect on proportion of ^{203}Hg associated with particulates. The acidified tubes (B and C) had a slightly higher proportion of the ^{203}Hg associated with the particulates than the control pH tubes (A) although A tubes overall were not different from C tubes (Table 10).

The average half-time of disappearance of ^{203}Hg determined in this study of 10.8 days was similar to that reported by other workers. Half-time of disappearance of ^{203}Hg from the epilimnion of an entire lake, 224, also in the Experimental Lakes Area was 14.3 days (Hesslein et al. 1980). ^{203}Hg disappeared with half-time of about 13 days from 10 m diameter tubes in Lake 223, ELA (Schindler et al. 1980a) and about 17 days from 10 m diameter tubes in Clay Lake, northwestern Ontario (Rudd et al. 1980).

According to Hesslein et al. (1980), settling of particulate material is an important pathway of removing radioactive metals from the water column. The Se tubes (series 2) behaved in a manner consistent with this. The higher proportion of ^{203}Hg present on particulate materials in series 2 tubes was associated with the fastest half-removal times. The study of Rudd et al. (1980) also showed that in the presence of selenium (100 μg L^{-1}), ^{203}Hg was associated with particulates to a somewhat greater extent than without selenium. But the presence of selenium in the latter experiment was not associated with faster half-removal time for ^{203}Hg.

The relationship between a high proportion of particulate ^{203}Hg and short half-removal time is not demonstrated when acidic and non-acidic tubes are compared. Under acidic conditions (tubes B and C), the proportion of ^{203}Hg in the particulates was higher than in neutral (A) tubes, but the half-removal time was shortest in A tubes.

Schindler et al. (1980a) found no effect (at the 0.05 probability level) of pH on the proportion on ^{203}Hg associated with particulates in 10 m diameter tubes in Lake 223. Half-removal times for ^{203}Hg were 12.7 days at pH 6.8, 11.1 days at pH 5.7 and 16.3 days at pH 5.1. These few data are consistent with our result of longer half-removal times in acidified tubes than in near-neutral tubes. Jackson et al. (1980) speculate that mercury may be deposited in sediments as "humic" complexes whose assimilation into sediments is inhibited by acidification.

ELEVATED ZINC CONCENTRATIONS IN TUBES

An unexpected complication of this tube experiment was discovered in 1981 when identical tube design was used to study the effects of low pH on the bioaccumulation of unlabelled aluminum and cadmium on these small ecosystems (S. Lawrence, pers. comm.). Analysis of water in tubes in 1981 showed that the concentrations of zinc ranged from about 30 to 200 μg L^{-1} (Table 11) and tended to rise with time. In comparison, zinc concentrations in open lake water were <10 μg L^{-1}. The contamination may have arisen from the galvanized metal culverts positioned outside the tubes on the sediment (S. Lawrence, pers. comm.), from the tube fabric itself (R. Hesslein, pers. comm.), or from other possible sources such as the PVC tubing within the tubes, the wire mesh of the crayfish cages or rubber gloves (S. Lawrence, pers. comm.). Samples of water from tubes in 1980, but analyzed in 1981, contained 20 to 250 μg L^{-1} zinc. In 1981, precautions were taken during sampling of tube water to avoid contamination by zinc and the 1981 values are considered to be more reliable.

As a possible indication of whether the zinc present in tube water in 1980 could have affected the bioaccumulation of the isotopes, tissues of crayfish from the tubes in 1981 were analyzed for zinc content. Tissues from several crayfish were pooled in some cases to provide sufficient tissue for metal analysis. Zinc concentrations in the hepatopancreas of crayfish from the tubes in 1981 were 1.5 to 8 times higher, and in green gland, 2 to 3 times higher, than in tissues from crayfish freshly-collected from Lake 239. There was no difference in zinc concentration in abdominal muscle in the two groups of crayfish (Table 11).

Bryan (1967) reported that the crayfish *Austropotamobius pallipes pallipes* held in 0.1% sea water containing less than 4 μg/L zinc had average zinc concentrations of 109, 11.5 and 7 μg^{-1} wet weight in hepatopancreas, abdominal muscle and green gland, respectively. Bryon (1967) also found that in the presence of elevated external zinc concentrations, zinc concentration increased in the hepatopancreas but not in the abdominal muscle.

Thus, there is evidence to suggest that the crayfish in tubes in 1980 would have absorbed zinc and accumulated it in some tissues to higher than normal levels. The effect of high external zinc or higher than normal zinc concentrations in body tissues on the uptake and accumulation of ^{203}Hg by the crayfish is unknown.

ACCUMULATION OF ^{203}Hg BY CRAYFISH TISSUES

^{203}Hg (cpm g^{-1}) inreased with time in all tissues even though ^{203}Hg (cpm L^{-1}) in water decreased exponentially with time. Mean bioconcentration factors were higher on day 35 than on day 7 in every case and for half the tissues were higher on day 35 than on day 15 (Table 12).

^{203}Hg (cpm, whole tissue) was accumulated to different extents by the seven crayfish tissues. Generally, the order was hepatopancreas > gills > gut > green glands > carapace > ovary > abdominal muscle. Order of concen-

Table 11. Concentration of zinc ($\mu g\ g^{-1}$ wet weight tissue) in three tissues from crayfish held in tubes in Lake 302 in 1981 and from 20 crayfish freshly-collected from Lake 239. Hep = hepatopancreas; mus = abdominal muscle; gr gl = green gland.

Experimental conditions	Zn conc. in water $\mu g\ L^{-1}$	Tissue	No. of crayfish	No. of chemical analyses	Zn conc. in tissue ($\bar{x}$ ± S.E.)
Freshly collected					
	<10	hep	20	16	49.9 ± 3.7
		mus	20	16	16.5 ± 0.63
		gr gl	19	1	35
Crayfish held in tubes					
40 $\mu g\ L^{-1}$ Al, pH 6.7	180	hep	9	1	223
		mus	9	1	16
		gr gl	14	1	85
40 $\mu g\ L^{-1}$ Al, pH 5.3	20	hep	5	1	91
		mus	5	1	18
		gr gl	pooled with 40 $\mu g\ L^{-1}$ Al, pH 6.7		
No metal addition, pH 6.7	110	hep	14	2	332
		mus	14	2	17
		gr gl	14	1	72
No metal addition, pH 5.3	135	hep	10	2	94
		mus	10	2	18
		gr gl	10	1	62
1 $\mu g\ L^{-1}$ Cd, pH 6.7	50	hep	15	2	214
		mus	15	2	16
		gr gl	15	1	75
1 $\mu g\ L^{-1}$ Cd, pH 5.3	85	hep	13	2	156
		mus	13	2	16
		gr gl	13	1	69
3 $\mu g\ L^{-1}$ Cd, pH 6.7	115	hep	12	2	301
		mus	12	2	18
		gr gl	12	1	65
3 $\mu g\ L^{-1}$ Cd, pH 5.3	150	hep	16	2	302
		mus	16	2	16
		gr gl	16	1	73

Table 12. Average bioconcentration factors ($\bar{x}$ ± S.E.) for Hg-203 in seven tissues at three sampling times. Data from series 1 to 5 are pooled. Lines connect pairs which are different at the 0.01 level using Tukey's ω procedure. All other pairs are not different at the 0.05 level.

	Days after addition of isotopes		
Tissue	7	15	35
Carapace	22.2 ± 3.2	56.5 ± 6.4	52.7 ± 6.2
Hepatopancreas	147.9 ± 36.3	265.3 ± 40.0	403.1 ± 46.9
Gills	207.4 ± 42.4	258.1 ± 39.0	346.0 ± 40.3
Green glands	336.8 ± 56.1	475.1 ± 65.0	873.1 ± 157.7
Ovary	27.1 ± 6.0	44.3 ± 8.5	44.1 ± 5.3
Gut	48.0 ± 11.1	146.0 ± 20.8	139.0 ± 16.6
Abdominal muscle	4.0 ± 0.8	8.4 ± 1.2	11.3 ± 1.3

tration of ^{203}Hg (cpm g^{-1} wet weight tissue) was similar: green glands > hepatopancreas > gills > gut > carapace > ovary > abdominal muscle (Figs. 1 to 6). Although green glands showed the highest rank order in concentration they were intermediate in total accumulation because they weighed 10 to 30 times less than the other tissues.

These results and other studies on the accumulation of metals (Cd, Cu, Pb and Zn) by *O. virilis* (Anderson and Brower 1978; Leonhard 1979), indicate that hepatopancreas and gills are the main sites of metal accumulation. Thus, it seems reasonable to select hepatopancreas or gill as target tissues for assessing metal accumulation in crayfish. On the other hand, Hamilton (1972) found the highest concentrations of mercury in crayfish to be in abdominal muscle when populations were exposed chronically to elevated mercury levels. Possibly mercury is concentrated early during exposure in the hepatopancreas and longer times of exposure are required for slower accumulation by the muscle.

EFFECT OF LOW pH

In some cases, average bioconcentration factors for ^{203}Hg in the seven tissues were lower in crayfish from the tubes at pH 5.4 or 5.0 than at pH 6.7 (Table 13). Thus, low pH appears to retard accumulation of ^{203}Hg at least in some conditions.

The inhibition of the uptake of cations by crayfish in low pH has been reported. Below pH 6.0, Na+ absorption by the crayfish *Astacus pallipes* is inhibited (Shaw 1960). pH below 5.75 progressively inhibits uptake of Ca^{++} by crayfish during postmolt (Malley 1980). Two possible hypotheses are suggested here to explain the retardation of ^{203}Hg uptake by low pH. Either ^{203}Hg is in cationic form and its uptake is inhibited by low external pH, or, alternatively, the tendency for ^{203}Hg to bind to particulates to a greater extent at low pH may make the ^{203}Hg less available for uptake by the crayfish.

EFFECT OF SELENIUM

Selenium had the most pronounced effect of all the treatments on the bioaccumulation of ^{203}Hg. The addition of selenium to tubes in series 2 in many cases significantly decreased the bioaccumulation of ^{203}Hg in all tissues at each sampling time when these crayfish were compared with those from series 1, 3, 4 or 5 (Table 14). In addition, concentrations of ^{203}Hg in most tissues of crayfish from tube 2A vs 1A and 5A, tube 2B vs 1B and 5B and tube 2C vs 1C and 5C are shown in Figs. 2 to 4. These results have clearly demonstrated that selenium reduced the accumulation of ^{203}Hg by most crayfish tissues under normal and acidic conditions.

Rudd et al. (1980) found that selenium appeared to retard the rate of ^{203}Hg accumulation by fish, crayfish and haptobenthos in the 10 m diameter tubes in Clay Lake, Ontario which has been severely contaminated by mercury for more than a decade. These results led Rudd et al. (1980) to suggest that a concentration of 10 μg L^{-1} of selenium could be used to ameliorate the mercury pollution problem in freshwater ecosystems. Our data support the finding of Rudd et al. (1980), and also indicate that selenium retards

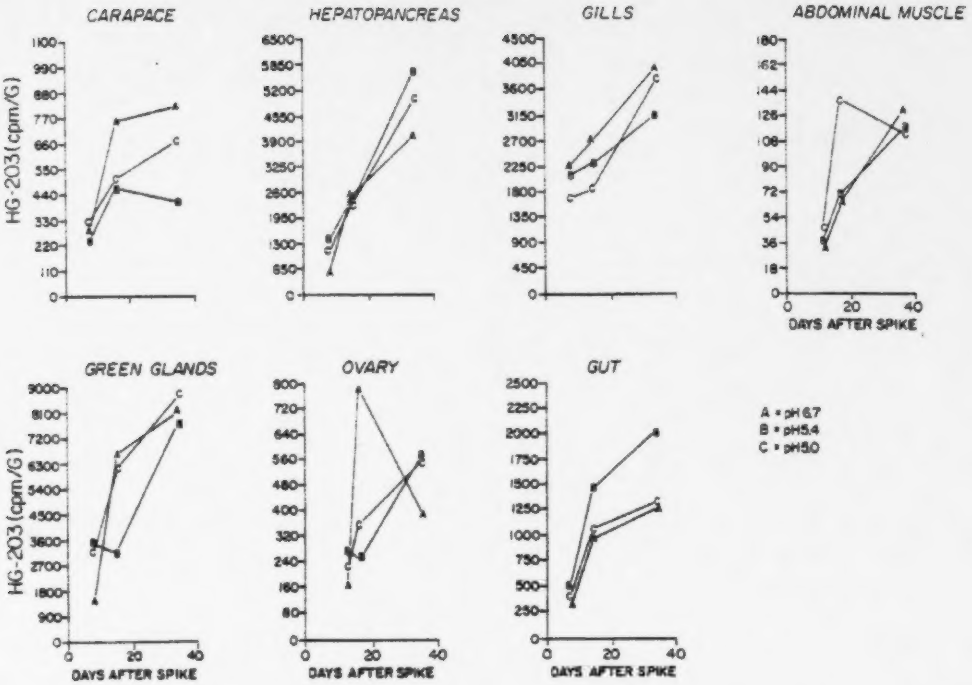

Fig. 1. Bioaccumulation of Hg-203 (cpm g^{-1} wet weight) by seven tissues of crayfish from tubes at pH 6.7, 5.4 and 5.0. A = tubes 1A + 5A, B = 1B + 5B, C = 1C + 5C. Each point represents the mean of 5 to 8 individuals.

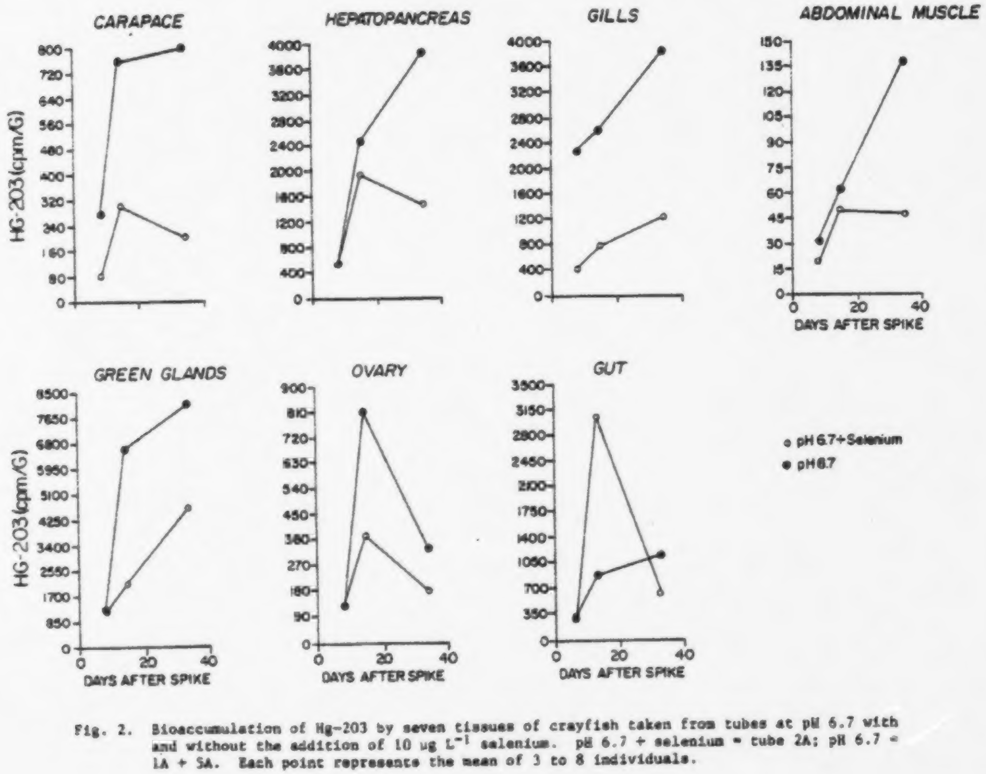

Fig. 2. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes at pH 6.7 with and without the addition of 10 µg L^{-1} selenium. pH 6.7 + selenium = tube 2A; pH 6.7 = 1A + 5A. Each point represents the mean of 3 to 8 individuals.

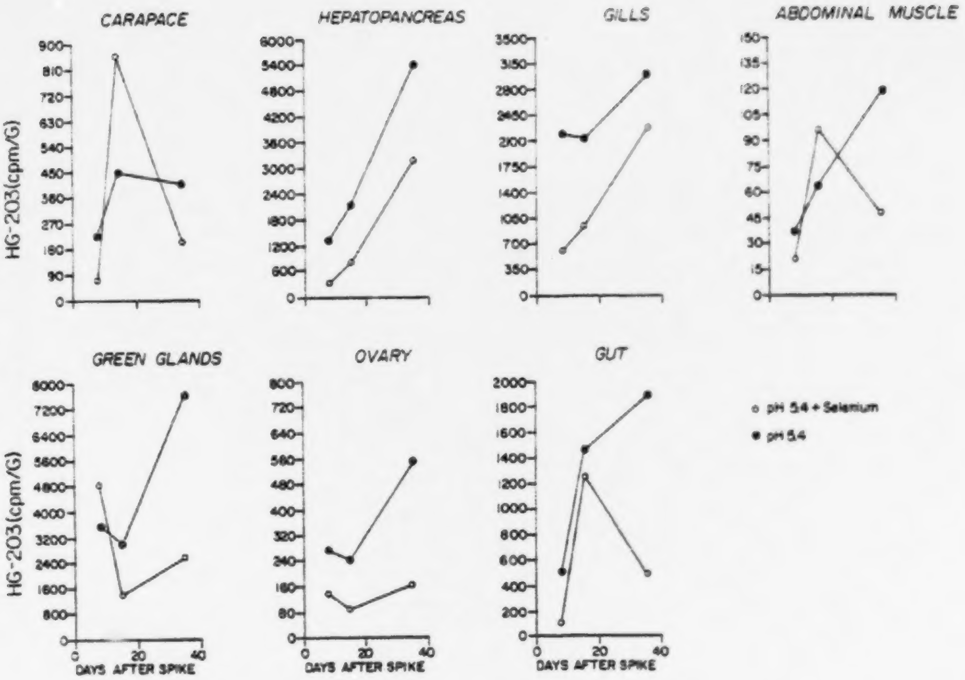

Fig. 3. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes at pH 5.4 with and without the addition of 10 ug L^{-1} selenium. pH 5.4 + selenium = tube 2B; pH 5.4 = 1B + 5B. Each point represents the mean of 3 to 8 individuals.

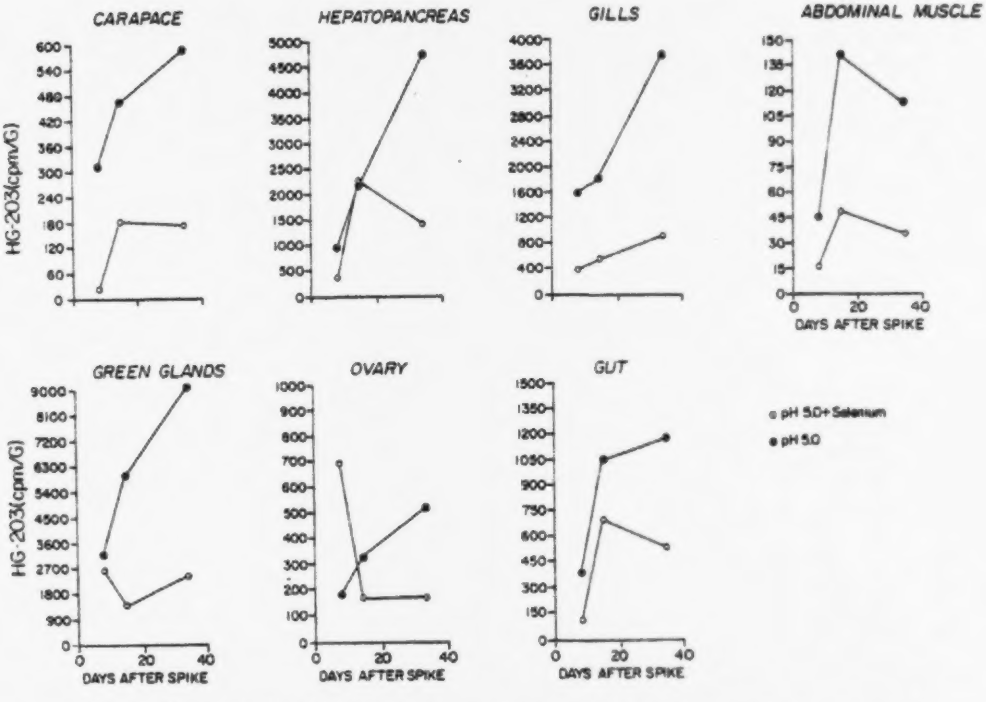

Fig. 4. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes at pH of 5.0 with and without the addition of 10 ug L^{-1} selenium. pH 5.0 + selenium = tube 2C; pH 5.0 = 1C + 5C. Each point represents 3 to 8 individuals.

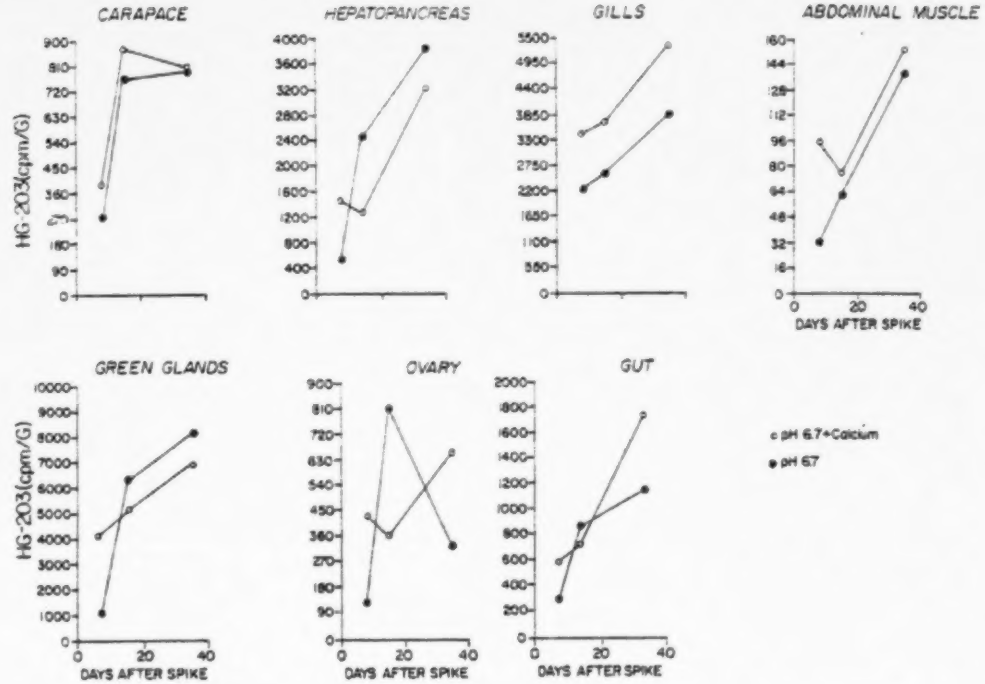

Fig. 5. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes held at average lake pH of 6.7 with and without the addition of 15 mg L^{-1} calcium. pH 6.7 + calcium = 4A; pH 6.7 = 1A + 5A. Each point represents 3 to 8 individuals.

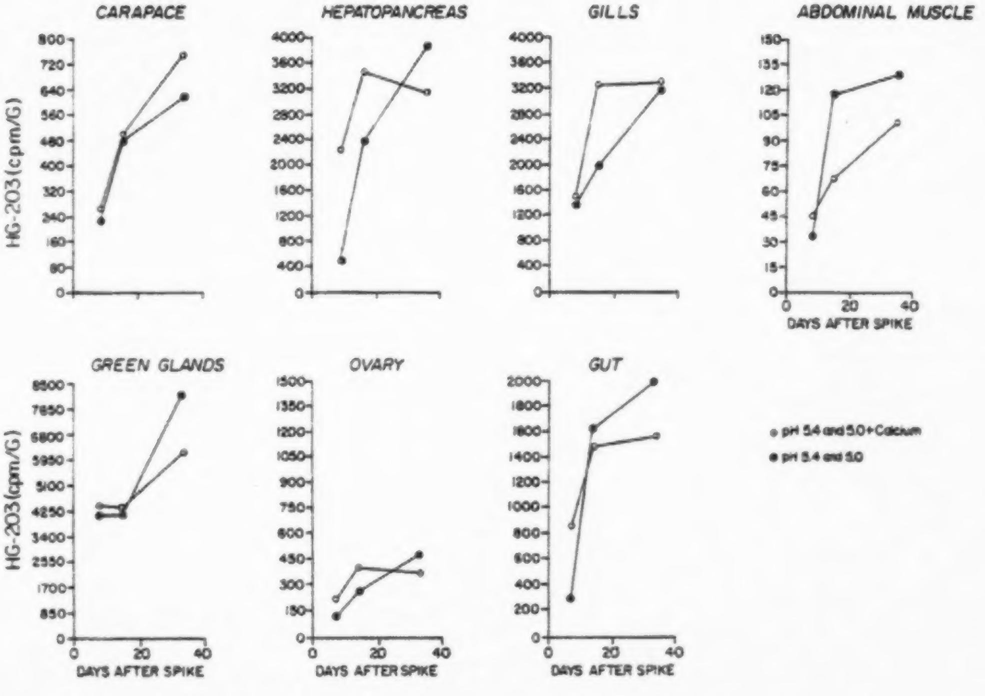

Fig. 6. Bioaccumulation of Hg-203 by seven tissues of crayfish taken from tubes at pH 5.4 and 5.0 with and without the addition of 5 and 15 mg L^{-1} of calcium. pH 5.4 and 5.0 + calcium = tubes 3B + 3C + 4B + 4C; pH 5.4 and 5.0 = 1B + 1C + 5B + 5C. Each point

Table 13. Average bioconcentration factors ($\bar{x}$ ± S.E.) for Hg-203 in seven tissues in three pH's at three sampling times. Data from series 1 to 5 are pooled. Lines connect pairs which are different at the 0.05 or 0.01 level using Tukey's ω procedure. All other pairs are not different at the 0.05 level.

Day after addition of isotopes	Tissue	Tube A	 B	 C
7	carapace	26.6 ± 7.5	19.7 ± 3.4	20.4 ± 5.6
15		65.8 ± 16.0	57.9 ± 16.5	45.8 ± 8.5
35		66.3 ± 14.0	44.5 ± 7.5	47.4 ± 9.4
7	hepatopancreas	136.4 ± 56.3	213.4 ± 93.0	93.8 ± 14.7
15		233.8 ± 45.2	344.9 ± 101.1	217.2 ± 44.8
35		332.3 ± 81.9	469.3 ± 66.6	407.7 ± 97.9
7	gills	294.5 ± 96.3	221.1 ± 67.0	108.2 ± 26.1
15		295.4 ± 69.5	309.8 ± 84.4	169.2 ± 32.0
35		393.8 ± 87.0	336.6 ± 63.1	307.6 ± 67.6
7	green glands	211.9 ± 53.9	355.0 ± 66.3	443.4 ± 137.3
15		593.9 ± 141.0	356.1 ± 88.5	475.2 ± 97.3
35		1291.9 ± 401.7	623.3 ± 131.0	704.3 ± 126.8
7	ovary	26.4 ± 9.3	26.2 ± 10.7	28.7 ± 13.1
15		60.4 ± 20.8	35.3 ± 11.1	37.3 ± 10.0
35		44.3 ± 11.1	47.4 ± 10.3	40.6 ± 7.4
7	gut	45.7 ± 18.2	69.8 ± 26.4	28.3 ± 6.6
15		154.1 ± 45.2	189.6 ± 27.8	94.5 ± 23.5
35		129.5 ± 23.8	168.2 ± 36.4	119.2 ± 26.0
7	abdominal muscle	5.2 ± 2.3	3.2 ± 0.5	3.5 ± 1.0
15		7.2 ± 1.0	8.5 ± 1.2	9.6 ± 3.3
35		13.1 ± 2.6	10.7 ± 2.2	10.1 ± 2.0

Table 14. Average bioconcentration factors (x̄ ± S.E.) for Hg-203 in seven tissues in 5 tube series. Data from tubes A, B and C are pooled. Lines connect pairs which are different at the 0.05 or 0.01 level using Tukey's ω procedure. All other pairs are not different at the 0.05 level.

Day after addition of isotopes	Tissue	Series 1	2	3	4	5
7	carapace	19.2 ± 5.4	5.8 ± 1.5	30.3 ± 6.6	25.1 ± 6.7	31.0 ± 4.7
15		50.8 ± 5.0	36.7 ± 18.9	66.8 ± 3.8	60.1 ± 20.7	66.1 ± 18.6
35		73.0 ± 14.3	19.1 ± 0.5	61.4 ± 2.6	60.0 ± 17.2	50.1 ± 4.1
7	hepatopancreas	37.5 ± 15.2	73.1 ± 34.7	324.8 ± 120.8	158.8 ± 39.9	145.4 ± 63.2
15		294.5 ± 49.7	174.0 ± 53.8	395.9 ± 94.1	274.1 ± 148.1	187.9 ± 57.5
35		511.9 ± 142.8	197.5 ± 45.1	464.7 ± 80.0	357.1 ± 45.6	484.1 ± 113.0
7	gills	134.0 ± 23.4	46.6 ± 3.6	286.5 ± 119.8	208.9 ± 94.1	363.5 ± 90.2
15		206.2 ± 18.1	76.6 ± 5.4	370.5 ± 55	391.1 ± 126.0	246.2 ± 30.0
35		325.7 ± 73.2	144.9 ± 30.2	469.0 ± 13.6	386.1 ± 121.0	404.4 ± 72.8
7	green glands	320.7 ± 135.7	303.6 ± 76.5	495.3 ± 240.6	373.5 ± 28.4	190.8 ± 49.5
15		424.8 ± 155.2	166.0 ± 24.8	666.0 ± 58.1	463.8 ± 109.8	665.0 ± 154.4

Table 14. Cont'd.

35		712.0 ± 158.5	322.3 ± 68.0	1129.3 ± 359.1	1248.4 ± 662.6	953.7 ± 41.0
7	ovary	10.7 ± 4.4	35.7 ± 22.4	21.1 ± 5.1	30.4 ± 14.0	37.6 ± 16.0
15		68.3 ± 35.5	21.9 ± 8.5	69.6 ± 3.4	31.3 ± 9.1	30.6 ± 6.4
35		44.1 ± 8.5	17.5 ± 1.2	54.4 ± 4.0	47.8 ± 15.4	56.5 ± 12.1
7	gut	29.0 ± 8.9	14.0 ± 2.4	106.0 ± 34.1	52.8 ± 20.2	37.6 ± 11.3
15		158.5 ± 22.3	165.3 ± 69.0	192.1 ± 52.4	128.3 ± 51.5	86.0 ± 34.0
35		183.3 ± 30.3	57.4 ± 6.1	152.4 ± 13	182.0 ± 41.0	120.1 ± 36.9
7	abdominal muscle	3.3 ± .9	1.8 ± .1	5.8 ± 1.7	6.1 ± 3.5	2.8 ± .7
15		10.7 ± 5.9	6.4 ± 1.3	9.5 ± 1.0	6.9 ± 1.3	8.5 ± 1.1
35		12.6 ± 1.5	4.3 ± .2	15.4 ± .2	10.8 ± 3.5	13.2 ± 2.1

^{203}Hg accumulation by crayfish under acidic as well as neutral conditions.

The higher proportion of ^{203}Hg associated with particulates in the selenium tubes and the faster half-time of disappearance suggests that selenium decreased bioaccumulation of ^{203}Hg by the crayfish by physically removing the isotope faster from the water column. Therefore, less ^{203}Hg was available for uptake by crayfish.

EFFECT OF ELEVATED CALCIUM CONCENTRATIONS

The addition of calcium to tubes to bring concentration to 5 or 15 mg L^{-1} generally did not affect bioaccumulation of ^{203}Hg by crayfish at normal lake pH of 6.7 (Fig. 5) nor at lower pH's (Fig. 6). Significant differences in mean bioconcentration factors between tubes 3 or 4 and 1 or 5 were rare and in these cases the tissues from the tubes with elevated calcium displayed higher concentration of ^{203}Hg, not lower as expected (Table 14).

High calcium concentration in lake waters was associated with lower accumulation (Cd, Cu and Hg) by fish (McFarlane and Franzin 1980). The highest concentration of Hg in fish muscle and liver was found to correspond with the lowest calcium concentration in a number of lakes and marshes in the Parry Sound area, Ontario, having calcium concentrations ranging from 2 to 25 mg L^{-1} (C. Wren, pers. comm.). However, although we increased the calcium concentrations 3x and 10x over the background level, there was no indication that elevated calcium concentrations reduced ^{203}Hg accumulation by crayfish. Preliminary results indicate that the presence of 5 and 15 mg L^{-1} of calcium also had no consistent effect on ^{203}Hg accumulation by white suckers (J. Klaverkamp, unpubl. data). Therefore, the trend of high calcium concentration and low mercury accumulation may not be applicable to all circumstances, at least not in the case of the crayfish from our study.

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METHODOLOGY FOR ASSESSING TOXICITY AVOIDANCE

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HADJINICOLAOU, J. and L.D. SPRAGGS. 1981. Methodology for assessing toxicity avoidance. Can. Tech. Rep. Fish. Aquat. Sci.

The design and implementation of a channel designed to perform dynamic avoidance testing of industrial effluents is discussed. An existing 9 m long hydraulics channel has been modified and appropriate holding tanks added to give an innovative new experimental tool in assessing the environmental impact of industrial effluents.

Prior to designing the channel an exhaustive literature survey was conducted to identify the types of experimental apparatus used in the past. In addition, the parameters associated with avoidance were enumerated and characterized as to their importance in avoidance experimentation. From the synthesis of the data gathered in the literature survey, an experimental apparatus was designed and an experimental procedure developed.

The objective of the present paper is to discuss the experimental equipment and the methodology used in the experiments. The rationale used in designing the methodology will be presented. The method of data acquisition and subsequent analysis will be discussed to give a general appreciation of the techniques being used to determine the avoidance. Preliminary results from testing the apparatus will be presented for subsequent discussion in conjunction with the rationale used in developing the new techniques described in the paper.

Key words: Toxicity assessment, industrial effluent.

HADJINICOLAOU, J. and L.D. SPRAGGS. 1981. Methodology for assessing toxicity avoidance. Can. Tech. Rep. Fish. Aquat. Sci.

La conception et l'utilisation d'un canal destiné à l'analyse dynamique de l'évitement d'effluents industriels sont discutées. La modification d'un canal existant, long de 9 mètres, ainsi que l'addition de bassins de "retenu" ont créé un outil original pour évaluer l'impact d'effluents industriels sur l'environnement.

Une étude complète a été conduite avant la conception du canal, pour identifier les différents types d'équipements expérimentaux utilisés jusqu'à présent. Les paramètres associés à l'évitement y sont énumérés et évalués quant à leur importance.

En se basant sur la revue de littérature existante, un protocole d'expérience a été développé et un appareillage conçu. L'objectif de cet article est de traiter de cette méthodologie, de sa logique et de l'appareillage. La méthode d'acquisition des données et leur analyse est discutée de manière à fournir une appréciation générale des techniques utilisées pour déterminer l'évitement. Les résultats préliminaires du test de l'appareillage sont présentés dans le but d'établir une discussion en conjonction avec la logique utilisée pour le développement de ces nouvelles techniques.

INTRODUCTION

Until recently, aquatic biologists engaged in research in government and academic institutions focused on ecological studies of lakes and rivers, or an evaluation of LC_{50} for various species and isolated chemicals. Conversely, environmental engineers were primarily concerned with stereotype designs of wastewater treatment plants for the removal of BOD and suspended solids. If the two disciplines met, it was usually unrelated to professional interests or activities.

In the past few years, however, the picture has undergone a substantial change. An increasing number of industries and regulatory agencies are attempting to relate pollution or discharges to the overall effect on the aquatic environment rather than restricted specific effects. These facts have led to a professional linkage between the engineer responsible for removing or modifying the pollutant and the biologist responsible for defining its effect on ecological systems.

The toxicity of effluents has acute or long-term effects on the aquatic life. Mobile aquatic organisms may often exhibit sensitive behavioural changes to the toxic pollutants (i.e., avoidance). The existing "industrial pollution control" recommends the use of acute bioassays in order to fulfill their particular water quality objectives.

This paper will therefore outline a methodology for assessing the reaction of a particular fish to an industrial effluent. By determining the behavioural change a value judgement can be made as to the potential impact of discharging the effluent into a natural waterway. It is not the objective of this paper to definitively discuss results but rather to present the methodology for scrutiny and appraisal.

The need for a new methodology emerges from the fact that acute lethal bioassays do not represent the actual total phenomena (sublethal and chronic effects) and also from the fact that industries discharge toxic effluents into natural water bodies often in an inadequate manner, without any consideration for the existing ecosystems.

Toxicity Avoidance - Summary of Previous Work —— Avoidance-preference tests provide information on the ability of aquatic organisms to detect lethal and sublethal concentrations of toxicants and to respond to the toxicant by moving into the toxicants or into non-polluted water. The rationale for testing the direct response to toxicants are to determine:

(a) whether the species can detect the toxicant, and
(b) if so, whether preference for the toxicant will render it actually more hazardous, or avoidance may provide a chance for escape.

Since 1913, six different designs of preference tanks have been introduced to study avoidance-preference. Beginning in 1913, Shelford and Allee introduced the first preference tank. This tank design was followed by Hoglund in 1953, Lindahl and Marcstrom in 1958 and Kleerekoper and Mogensen in 1963. In each of these designs the basic concept is the same, in that the tank allows several different streams of varying concentration to flow side by side. The time spent by the fish in each stream is visually recorded and the preference or avoidance calculated.

However, other researchers found that exposing a specimen to a gradient of several concentrations may alter the response due to toxic action already taking place during the course of the test. Therefore after 1964 new test designs appeared in the literature which provided for a reliable separation between pure and toxicant-contained water, with a device for tracing and documenting the specimen's position into and out of the toxicant accurately.

Hansen in 1969, and 1972-1973, and Folmar in 1976 used the single and double Y maze in which a toxicant is introduced into one Y-shaped arm and pure water into the other. The two media are drained into the circular holding area. The disadvantages of the double Y maze are that aquatic organisms may be affected by eddies and areas of no current in the circular holding area and toxicant concentration in the circular holding area may not be homogeneous because of the turbulence.

Sprague in 1964 modified the idea of Jones in 1947, using the counter-current tube and later Scherer and Nowak in 1973 used the rectangular counter-current system. A toxicant is introduced into one end of the tube (or system) and water into the other end. The two media meet at the centre of the tube creating a toxicant-water interface. One possible disadvantage of both counter-current systems is that the change in current direction at the drain may effect the behaviour of aquatic organisms, thus reducing the frequency of interface crossings.

Weslake and Lubinski in 1976 designed the open field system. The same system has been used in 1977 by Sprague and Weslake with very small modification. In the open field system the toxicant and the water flow side by side in the same direction across a 50 x 50 cm observation area. The position of the fish is recorded with respect to 16 square regions on the tank bottom. Two main disadvantages of this particular design was the difficulty to maintain a strict interface between the toxicant and the water and the difficulty of relating the lab results to *in situ* discharges. The sharp gradient in the laboratory apparatus is unlike those found in the field.

More recently researchers have attempted to develop techniques for *in situ* toxicity studies. The most advanced apparatus was developed by Birtwell (1977) "to study the behaviour of fish in shallow, stratified waters". This technique is particularly suited to determining the avoidance or preference by fish of water quality changes in both polluted and unpolluted situations.

Most of the previous studies have been conducted for individual chemicals (chlorine, ammonia, heavy metals, etc.). However, current studies have been directed towards the resolution of behavioural changes from actual industrial effluents. The present apparatus has been designed to handle either single chemicals or relatively large volumes of industrial effluents.

DESIGN AND SET-UP FACILITIES

A description of the components of the continuous flow system used in the present study will follow. Much of the equipment has been adapted from equipment already in place in the Civil Engineering Hydraulics Laboratory at McGill University. Figures 1 through 3 show the system schematically.

Water Treatment —— Water for the system is taken from the domestic supply of the City of Montreal. Prior to actual use it is treated to make it suitable for the fish to live in. Trout used in the study have continued to thrive and grow in the water having a growth rate of approximately 2 cm per month for the new (2 - 3.5 g) fish.

City water enters the system and passes immediately through an activated carbon tower (A.C.) to remove organics, amines, taste, colour, etc. The efficiency of the present A.C. is of the order of 95%. Because bacteria are not removed in the A.C. and may in fact reproduce rapidly, the water is subsequently passed through a water sterilizer (ultra-violet lights; U.V.). Finally, the water is sent to a large storage reservoir for holding and temperature adjustment.

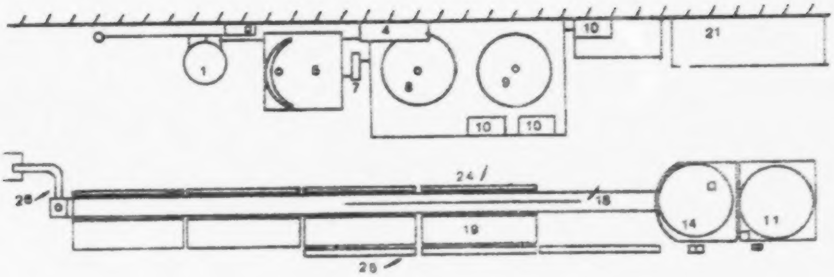
Temperature Control —— A submerged heating unit in the large storage reservoir, with a control panel having a magnetic contactor of 40 amps, a main disconnecting switch of 60 amps and an automatic temperature control unit provides a continuous temperature of 15°C in the system. In addition, in the summer cooling units are used to cool the water to 15°C.

Ten waterproof temperature probes have been strategically located throughout the system (channel, holding tanks, etc.) and connected to a manually operated multi-channel thermometer for quickly analyzing the temperature at any point in the system.

The Channel —— The experimental channel is 9.14 m long, 30 cm wide and has a continuous water elevation of 33 cm. It is divided into 5 sections of 1.8 m each. The first section has been built with plexiglass to allow for holes for jets and diffusers while the other four were built with glass. In the first section of the channel, 1.5 m from the front gate and 0.3 m from the second section, provision has been made for the injection of the effluents and dyes from the effluent tank.

A 6 mm plexiglass barrier with special holding devices has been placed in the centre of the channel from the mid-point of the first section through to the third section (4.2 m). This separation unit has been introduced to give the fish the opportunity to choose either the polluted or the non-polluted side of the channel.

Because it is desirable to have a three-dimensional analysis of the disposition of the fish in the channel, mirrors have been placed along one side of the channel. The mirrors are placed at an angle of 45° and when photographed from above both the horizontal projection and the vertical projection are shown on the screen. Consequently, any avoidance in either the lateral, horizontal or vertical direction will be recorded. Horizontal coverage is provided by the placement of four overlapping cameras along the length of the channel.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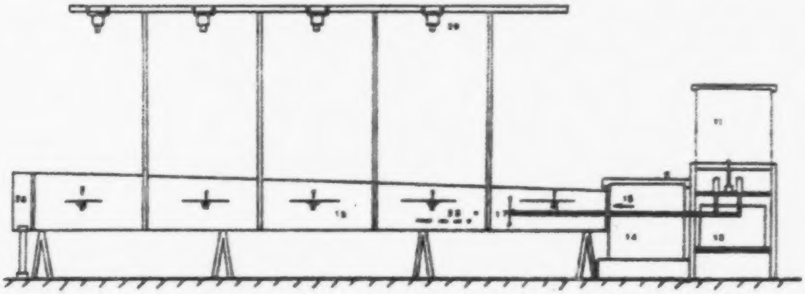
1	ACTIVATED CARBON UNIT	9	FISH HOLDING TANK C	19	MIRRORS
4	ULTRA VIOLET LIGHT	10	SMALL WATER COOLERS	21	VIDEO SYSTEM
5	STORAGE RESERVOIR	11	EFFLUENT TANK D	24	CHANNEL LIGHTING
7	MAIN PUMP	14	ACCLIMATION TANK E	25	SIGHT AND SOUND BARRIERS
8	FISH HOLDING TANK B	18	CENTER SEPARATION UNIT	26	END GATE AND DRAINAGE

FIGURE 1. PLAN VIEW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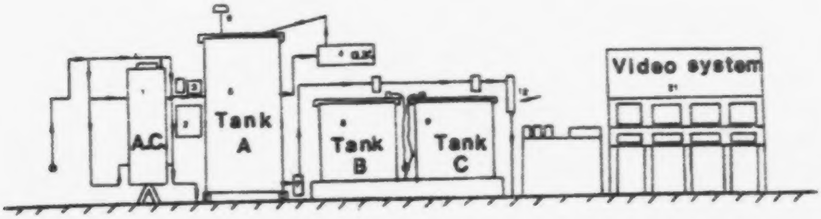
11	EFFLUENT STORAGE TANK	17	WALL INJECTION POINTS
13	LARGE COOLER	20	VIDEO CAMERAS
14	ACCLIMATION TANK E	23	CHANNEL POSITION INDICATORS
15	FRONT GATE TO CHANNEL	26	END GATE AND DRAINAGE
16	CHANNEL		

FIGURE 2. SIDE VIEW WITHOUT THE MIRRORS


A.C.
Tank
A
Tank
B
Tank
C
Video system
21
12

1	ACTIVATED CARBON UNIT	7	MAIN PUMP
2	HEATING CONTROL UNIT	8	FISH HOLDING TANK B
3	AUTOMATIC TEMPERATURE CONTROL	3	FISH HOLDING TANK C
5	STORAGE RESERVOIR	12	FLOWMETER
6	HEATING COIL	21	VIDEO SYSTEM

FIGURE 3. SIDE VIEW OF FISH HANDLING APPARATUS

The bottom and the one side of the channel are divided into 10 cm x 10 cm squares with black stripes on one side of every section. Each of these squares has been colour coded horizontally and vertically to aid in the determination of the behaviour of the fish in the channel.

Because the light reaching the iris of the cameras was not adequate and because the picture in the monitors from the mirrors must be clear it was decided to put fluorescent lights along the length of the channel. These lights are on for 12 hours each day.

Large barriers have been located along the exposed side of the channel to eliminate both noise and visual disturbances while the experiments are in progress.

A plexiglass box has been built at the downstream end of the channel to simulate the flow of water in a river while the perforated end gate allows the passage of water. The diameter of the holes and width of slots in the end gate is too small to allow the fish to pass through. The end gate is removeable to allow for rapid draining and easy cleaning of the channel.

The modifications and extensions to the facility were completed and tests were performed to ensure that the facility was functioning correctly. Initial testing indicates that the facility will become a viable diagnostic tool in the future.

The design of the channel fits the needs of the innovative approach and the advantages of the previous systems;

1. provide possibilities for vertical and horizontal analysis;
2. use video system with the possibility to store the results;
3. use different injection systems;
4. represent the natural phenomenon better;
5. use both total separation and side by side separation of water and different toxicants.

The physical set-up as shown in Figure 1 has been implemented and tested for hydraulic properties. Considerable work was required to achieve parallel flow and to construct a system which closely resembled field conditions.

Data Acquisition — Four cameras have been mounted on moveable trolleys above the channel. Each of the cameras can cover approximately 1.8 m along the channel giving total coverage to the experimental area of the channel. Output from the cameras is viewed on monitors and then saved on video recording machines.

The signals from the four cameras are monitored with a video monitor for each section of the channel. Simultaneously long play video recorders are used to record the video signal for later analysis. One of the recorders has audio dubbing capabilities to allow audio documentation of archive tapes.

DATA ANALYSIS

Four video cassettes recorders are used to record the events of every experiment; both vertically and horizontally. If the duration of the experiment is 2 hours, they record continuously; if the duration is 8 hours they record in very precisely prescribed intervals, covering the 8 hours.

After the experiment an analysis is made of the tapes at 5-min intervals. The recorders are stopped and a count is made of the distribution of fish in the channel. The results are transferred manually to a computer storage media and subsequently analysed statistically by using SAS on the McGill computer. To complete the test all the important portions of the tapes are written on archive tapes for future reference and comparison.

Limitations in the present analysis method are summarized as follows:

1. visual counting errors;
2. the analysis is time-consuming and slow;
3. more than one person is required.

A future modification to the present system is to computerize the analysis, using a new system that has been developed by EPA at the Rhode Island Environmental Research Laboratory. The so-called "Bug System" contains a video preprocessor (special interface), a mini computer, plotter, line printer and graphics console. The above system has been used for sublethal effects other than avoidance and according to EPA researchers this would be the first time that such a system would be used for avoidance studies.

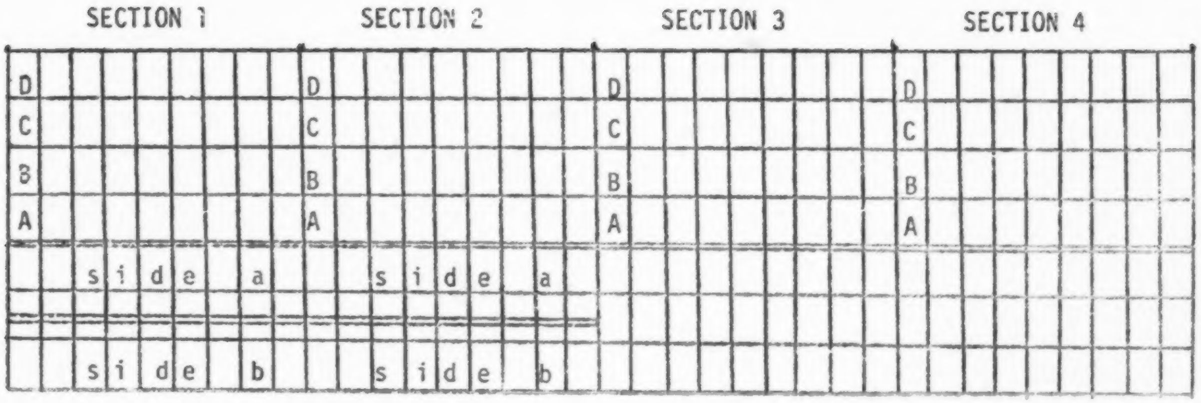
PRELIMINARY RESULTS

A. *General Disposition*

(a) *Without any pollutant* — Five tests have been conducted on different days and with different batches of fish to establish a general pattern of the disposition of fish, throughout the channel without any pollutant. The results are as follows:

Horizontal	Vertical	Lateral
Section 1 - 34.79%	Depth A - 48.45%	Side a - 49%
2 - 25.03%	B - 22.85%	Side b - 51%
3 - 22.16%	C - 28.65%	
4 - 18.02%		

The side distribution is almost even. There is no significant preference for Side a or b. The fish however prefer to swim more in the bottom (A), than in depths B and C. Also they have a tendency to use the horizontal sections with a decreasing preference as the section number increases (1,2,3,4). Figure 4 is a definition sketch of the channel showing the locations of the horizontal sections, the vertical depths and the lateral sides.


SECTION 1
SECTION 2
SECTION 3
SECTION 4
D
C
B
A
side a
side b

side part
or vertical
distribution

bottom part
or horizontal
or lateral
distribution

Horizontally -----→ top view - Sections 1, 2, 3, 4
Vertically -----→ through the mirrors - Depth A, B, C, D
Laterally -----→ top view - Sides a, b

FIGURE 4. VIEW OF THE T.V. MONITORS

(b) *With a clear water jet of 3.77 L/min in side a* — Five more tests have been conducted with a small clear water flow from one side of the channel, to evaluate the effect of a small jet on fish distribution. The results are as follows:

Horizontal	Vertical	Lateral
Section 1 - 32.46%	Depth A - 46.5%	Side a - 54%
2 - 28.22%	B - 20.78%	b - 46%
3 - 25.04%	C - 32.72%	
4 - 14.28%		

The jet (a multiple port diffuser located at depth B), causes a significant change in the distribution of the fish in the channel. Not only is there a 5% change in lateral preference for the jet but also a vertical migration of the fish to the level of the jet.

B. *Effect of Temperature* — The temperature gradient longitudinally in the channel is generally less than 0.5°C. Therefore, it was deemed necessary to run a battery of tests to determine if this gradient would significantly alter the test results. As a result six tests were conducted with influent temperature ranging between 12°C and 23°C. The results of these tests are given graphically in Figures 5 and 6.

It appears there is a pronounced avoidance reaction to high temperatures; especially for temperatures higher than 16°C. In the range between 16°C-25°C only one or two degrees difference has a tremendous effect on the distribution curve. For the low temperatures the effect is much smaller. From 10°C - 15°C there is a noticeable difference but much smaller than with high temperatures.

In a high temperature environment the fish prefer the coldest section, and in very low temperature the vertical disposition changes indicating that the temperature is not the most comfortable for them. For example, in 21°C - 22°C we have a high concentration in the end section (the coldest), and at 12°C a movement from depth A to depth B, whereas between 14°C-16°C the distribution tends to be even. Therefore, for the experiments the temperature will be held constant at a level of 14°C.

C. *Effect of Noise* — Four tests have been conducted with noise levels varying from 60-80 dBA. The horizontal distribution in almost all cases was the same, with a small difference in the vertical distribution, due to the expected normal trend to deeper water in the higher noise level. However, in general, the fish cannot detect noise levels. It is expected that the normal noise level in the lab will not be a factor in the research.

D. *Effect of Light Intensity* — Three experiments have been conducted to determine the effect of light intensity on fish behaviour:

(a) with very low light in dark environment
(b) with normal lights (day lights and side lights)
(c) high lights (changing different light intensities in different sections)

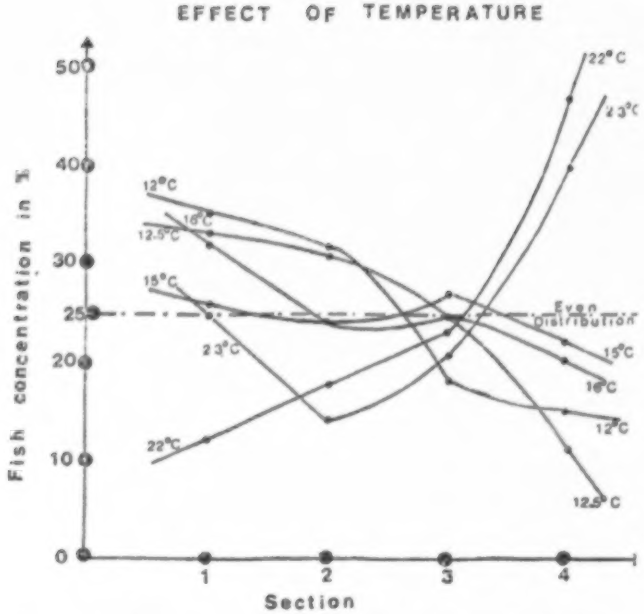


Fig. 5. Effect of temperature on horizontal distribution of fish in the channel.

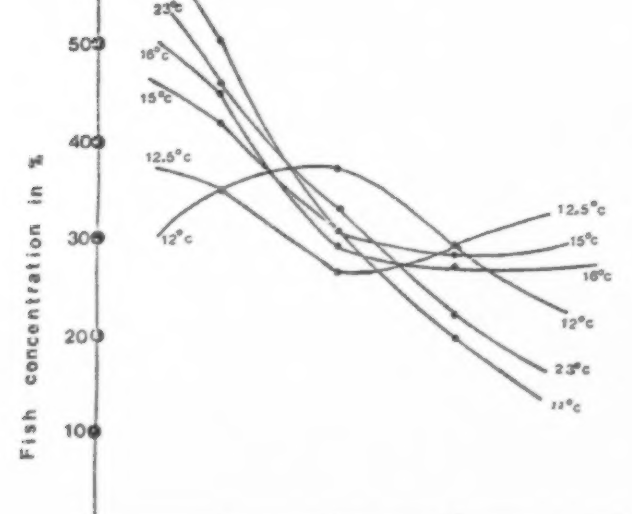


Fig. 6. Effect of temperature on vertical distribution of fish in the channel.

The results indicate that the intensity of light will not be a significant factor in the research as long as it is not erratic and as long as the lighting is constant along the length of the channel. The lighting intensity will be fixed to give the most favourable picture in the video system. Fluorescent lights were therefore installed along the entire length of the channel to give constant, reasonable lighting during the acclimation and experimentation.

E. *Experiments with the Reference Pollutant DSS* (dodecyl sodium sulfate) — DSS has been used extensively as a reference pollutant in industrial pollution bioassays, because of its almost stable value of LC_{50} in static toxicity tests (LC_{50} = 5-7 mg/L).

Seventeen tests have been conducted with DSS in eight different concentrations (1:400 LC_{50}, 1:200 LC_{50}, 1:100 LC_{50}, 1:40 LC_{50}, 1:32 LC_{50}, 1:8 LC_{50}, 1:4 LC_{50}, 1:1.3 LC_{50}) from both sides a and b, to evaluate the effect of DSS on the horizontal and vertical distribution and also to determine the avoidance curve.

In the horizontal direction there does not appear to be a significant change in the distribution in the four sections. The 5-10% variance with the unpolluted distribution curve is due to the injection system used (Fig. 7).

In the vertical direction the effect of the injection system is obvious and a significant trend from depths A to C appears (Fig. 8). The results indicate a 10% difference due to the injection system, and a 10% difference due to the DSS concentration up to approximately 1:300. After this concentration there is no significant change in the vertical distribution. In Figure 9 (using a log scale for DSS) the avoidance curve indicates that the fish prefer to swim in the polluted side for very low DSS concentrations (between 1:1000 and 1:100 LC_{50}). After the 1:100 LC_{50}, there is a slight tendency to avoid the pollutant and as they reach the lethal value, a 66% avoidance reaction appears.

CONCLUSIONS

A new system has been designed and implemented, which uses the advantages, and eliminates the disadvantages of previous avoidance systems while fulfilling the recent needs for toxicity avoidance studies, in the effort to link the gap between existing toxicity standards and actual damage under realistic environmental conditions. The first tests have shown that the system works exceptionally well. It is hoped that further experimental study, with the use of actual effluent discharges, will lead to a complete investigation of the overall problem.

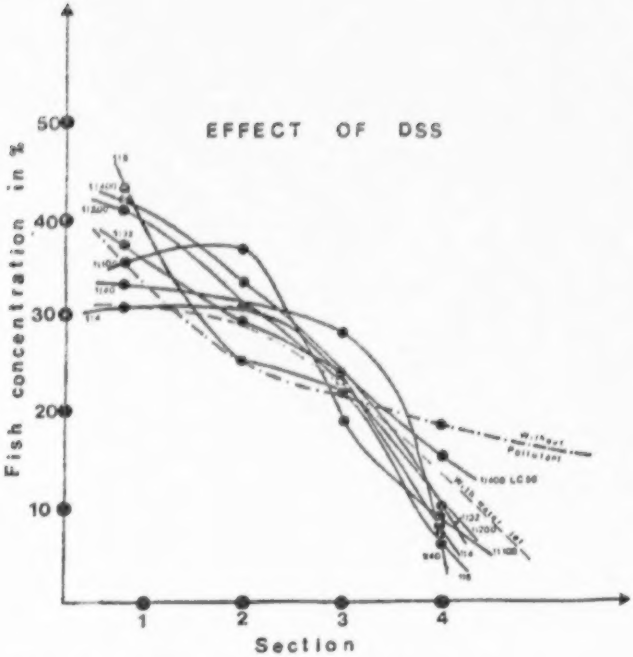


Fig. 7. Effect of DSS on horizontal distribution of fish in the channel.

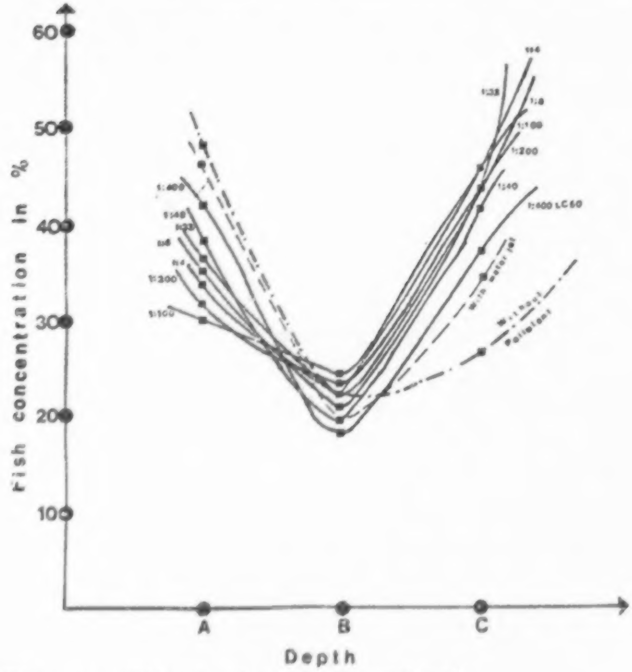


Fig. 8. Effect of DSS on vertical distribution of fish in the channel.

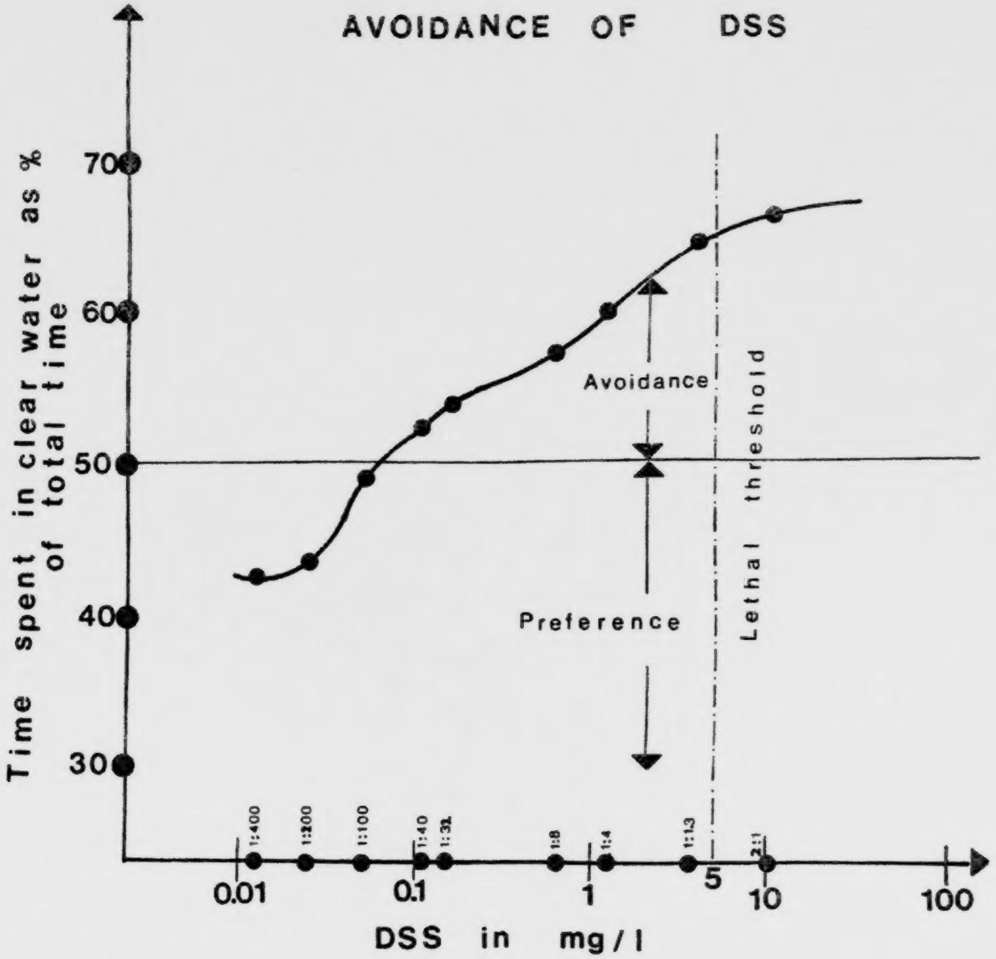


Fig. 9. Avoidance of DSS by fish.

ACKNOWLEDGMENTS

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EFFECT OF SELENIUM ON IMPOUNDED ZOOPLANKTON IN A MERCURY CONTAMINATED LAKE

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LAWRENCE, S.G., and M.H. HOLOKA. 1981. Effect of selenium on impounded zooplankton in a mercury contaminated lake. Can. Tech. Rep. Fish. Aquat. Sci.

The addition of selenium to fresh water contaminated by mercury has been suggested as an ameliorative measure for the toxic effects of mercury. This study examined the toxicity of two concentrations of selenium to zooplankton in Clay Lake, Ontario, which has contained added mercury for more than a decade.

Small 20L impoundments incubated *in situ* for 10 days and large 1.5 x 10^5L impoundments situated in a bay of the lake and sampled seven times in 56 days were used to examine effects of 0.1 mg selenium/L. The short-term impoundments were also used to examine effects of 1.0 mg selenium/L. The zooplankton community as sampled was composed of rotifers, herbivorous and carnivorous cladocerans, and calanoid and cyclopoid copepods. Changes in zooplankton numbers and calculated dry weight biomass were examined.

In small impoundments which contained 0.1 mg Se/L, herbivorous cladoceran and calanoid copepod biomass were significantly less than in control impoundments. Total rotifer and cyclopoid copepod biomass and numbers were unaffected in these conditions. The biomass of *Keratella cochlearis* increased significantly in the presence of 0.1 mg Se/L. Numbers of cladocerans were significantly different at this concentration, but total numbers and numbers of other community components were not. In small impoundments which contained 1.0 mg Se/L, total zooplankton biomass and that of all components of the community except the cyclopoid copepods were significantly decreased when compared to those in control impoundments. Numbers of zooplanktors and those of all community components significantly decreased at this concentration. Changes were verified using ANOVA.

In a large impoundment which contained 0.1 mg Se/L, total zooplankton biomass and the biomass of all community components except cyclopoid copepods were reduced by 80-96% compared to that in a control impoundment. Numbers of zooplanktors were reduced by 66-99%.

It is concluded that each concentration of selenium is too high to serve as a safe ameliorative measure in this lake.

Key Words: Toxicity, biomass, zooplankton, bioassay, mercury, selenium.

LAWRENCE, S.G., and M.H. HOLOKA. 1981. Effect of selenium on impounded zooplankton in a mercury contaminated lake. Can. Tech. Rep. Fish. Aquat. Sci.

L'addition de sélénium à l'eau fraîche contaminée par le mercure a été suggérée comme mesure diminuant les effets toxiques du mercure. Cette étude examine la toxicité de deux concentrations de sélénium au zooplancton dans le lac Clay, Ontario, qui conient du mercure depuis plus de 10 ans.

Les effets d'une concentration de sélénium de 0.1 mg/ℓ ont été examinés dans de petits bassins de 20 ℓ incubés *in situ* pour 10 jours et de plus larges bassins de 1.5 x 10^5 ℓ situés dans une baie du lac et échantillonnés 7 fois en 56 jours. Les effets du sélénium à 1.0 mg/ℓ ont aussi été examinés dans les petits bassins. La communauté de zooplancton, telle qu'échantillonnée, comprend des rotifères, des cladocères herbivores et carnivores et des copépodes calanoïdes et cyclopoides. Les changements dans la quantité de zooplancton et dans le biomasse calculée en poids sec sont examinés.

Dans les petits bassins qui contiennent 0.1 mg Se/ℓ, la biomasse des cladocères herbivores et des copépodes calanoïdes est significativement plus petite que dans les bassins de contrôle. Le nombre et la biomasse totales des rotifères et des cyclopoïdes ne sont pas influencés par ces conditions. La biomasse de *Keratella cochlearis* augmente significativement en présence de 0.1 mg Se/ℓ. Le nombre de cladocères est significativement différent à cette concentration mais le nombre total et le nombre des autres membres de la communauté ne le sont pas. Dans les petits bassins qui contiennent 1.0 mg Se/ℓ, la biomasse totale du zooplancton et celle de tous les membres de la communauté excepté les copépodes cyclopoïdes diminuent significativement comparativement aux bassins de contrôle. La quantité de zooplancton et de tous les membres de la communauté diminue significativement à cette concentration. Les changements ont été vérifies en employant ANOVA.

Dans les grands bassins contenant 0.1 mg Se/ℓ, la biomasse totale du zooplancton et la biomasse de tous les membres de la communauté excepté les copépodes cyclopoïdes sont réduits de 80-96% comparativement aux bassins de contrôle. La quantité de zooplancton diminue de 66-99%.

Il est conclu que chacune de ces concentrations de sélénium est trop élevée pour servir de mesure d'amélioration dans ce lac.

INTRODUCTION

Contamination of freshwaters by mercury has been recognized as a global environmental and health hazard because of its concentration in higher food chain organisms likely to be consumed by man (Fagerstrom and Asell 1977). One of several methods suggested to ameliorate the effects of mercury in biota of waterbodies is the addition of selenium which reduces the rate of mercury accumulation in fish and crayfish (Rudd et al. 1980) and reduces the toxicity of mercury and methyl-mercury in various animal communities (Ganther et al. 1972, Sandholm et al. 1973, Sumino et al. 1977).

In 1978-80, a major Canadian research effort was directed at amelioration of problems presented by the entry of mercury at Dryden, Ontario into the English-Wabigoon River system (Jackson, 1980). Part of this effort examined the effects of selenium on uptake, distribution and accumulation of mercuric compounds in aquatic biota introduced to large impoundments placed in a lake (Rudd et al. 1980). Other work investigated the direct effects of selenium on aquatic organisms which already existed in an environment containing various forms of mercury. This paper presents data on the effects of selenium on impounded zooplankton communities native to a bay of mercury contaminated Clay Lake, Ontario (Armstrong and Hamilton 1973), a part of the English-Wabigoon River system.

MATERIALS AND METHODS

Small impoundments containing zooplankton were established as described by Marshall and Mellinger (1980), except that the carboys held 20 L lake water and were incubated at one depth only, 1.5 m. To ensure even distribution of zooplankton, 60 L volumes of lake water were taken from 0.5 - 1.5 m in the bay with a van Dorn sampler, thoroughly mixed and distributed. Zero, 0.1, or 1.0 mg/L selenium (as sodium selenite) was added to sets of three carboys which were then incubated on the bottom of the bay for 10 days in July - August, 1978. At the end of the incubation period, contents of each carboy were filtered through 73 μm pore nylon net and the zooplankton preserved in 4% formalin (final concentration). Zooplankton representing 20% of the total were taken in two aliquots for identification to species and counting. Biomass was calculated as in Lawrence (1980). One way analysis of variance was used to compare the zooplankton communities in the experimental and control sets of carboys.

Large impoundments (1.5×10^5 L) which enclosed natural lake water including the zooplankton community were placed in the bay in summer, 1978 (Rudd et al. 1980). ^{75}Selenium was added to these impoundments to monitor Se concentration. One impoundment received no further addition. Selenium (as sodium selenite) was added to another impoundment to bring the selenium concentration to 0.1 mg/L on 19 July. These impoundments were sampled for zooplankton on seven dates from 15 July to 9 September, 1978 using a transparent trap (Schindler 1969) equipped with a 73 μm pore nylon net filter. Zooplankton were taken from 0.5, 1.0 and 1.5 m and subsequently combined into a single sample. Zooplankton were identified and counted and biomass calculated as above. Zooplankton present in samples taken during the experimental period are listed in Table 1.

Table 1. Zooplankton species list, Clay Lake, 15 July-9 September 1978.

	Dominant[a]	Present[b]	Occasionally Seen
COPEPODS			
Cyclopoidea			
[c]Tropocyclops prasinus mexicanus	X		
[c]Cyclops bicuspidatus thomasi			X[d]
[c]C. vernalis			X[d]
Eucyclops agilis			X
[c]Mesocyclops edax			X[d]
Calanoidea			
Diaptomus oregonensis		X	
[c]D. minutis			X[d]
Epishura lacustris			X
Cladocera			
[c]Bosmina longirostris	X[d]		
[c]Diaphanosoma brachyurum	X[d]		
[c]Chydorus sphaericus		X[d]	
Alona rectangula			X
A. quadrangularis			X
[c]Ceriodaphnia reticulata			X
C. lacustris			X
Daphnia retrocurva			X
Graptoleberis sp.			X
[c]Leptodora kindti			X[d]
Pleuroxus procuris			X
[c]Sida crystallina			X
Rotifera			
[c]Keratella cochlearis	X		
[c]Ploesoma sp.	X		
[c]Trichocerca cylindrica	X		
Asplanchna sp.		X	
Conochilis sp.		X	
Hexarthra sp.		X	
[c]Kellicottia longispina		X	
[c]Polyarthra sp.		X	
Synchaeta sp.		X	
[c]Anuraeopsis sp.			X
Asplanchnopsis sp.			X
Brachionus sp.			X
[c]Colletheca sp.			X
[c]Euchlanis sp.			X
Filinia longiseta			X
Keratella quadrata			X
[c]Lecane sp.			X
Mytilina sp.			X
Pompholyx sp.			X

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[a] Represents >10% of the biomass during the experimental period.

[b] Represents <10% of the biomass, but is always present.

[c] Represented in the small impoundments.

[d] Of major importance in the small impoundments.

Selenium concentrations were measured on unfiltered samples from the small impoundments using the method of Vijan and Wood (1976); samples were acidified prior to analysis. ^{75}Se concentrations in the large impoundments were monitored according to Hesslein et al. (1980). Mercury concentrations in the lake and in the large impoundments were measured using flameless atomic absorption (Hendzel and Jamieson 1976).

RESULTS

Small impoundments

Using analysis of variance, no statistical difference was found among the three samples taken from any one set of carboys, control or experimental. Tables 2 and 3 show the effects of 0.1 and 1.0 mg/L selenium on various components of the zooplankton community using one way analysis of variance to compare the mean biomass or mean numbers of the control set to each of those treated with selenium. Total biomass and the biomass of all community components except adult and copepodite cyclopoid copepods were decreased in the presence of 1.0 mg/L selenium. Herbivorous cladocerans and calanoid copepods biomass decreased in 0.1 mg/L selenium. The total rotifer community biomass was not affected by 0.1 mg/L Se in this experiment, but *Keratella cochlearis* biomass increased. Numbers of cladocerans were significantly different in 0.1 mg/L Se, but changes in number of other components of the community were not significantly different. Actual concentrations of selenium in the water contained in the small impoundments are close to nominal concentrations (Table 2).

Large impoundments

Tables 4 and 5 show the average total and average proportional biomass/L and numbers/L in the large control and experimental impoundments throughout the experimental period. The experimental impoundment (0.1 mg/L Se) consistently contained less zooplankton biomass (Fig. 1) and fewer numbers (Fig. 2) when compared with those in the control impoundment except for the cyclopoid copepods. Total biomass and biomass of all community components except the cyclopoid copepods were affected by 0.1 mg/L Se. The rotifer community, considered over the experimental period, was also sensitive to this concentration. ^{75}Se water column concentration decreased linearly with a half-life of 56 days in the large control impoundment and 49 days in the experimental impoundment (Rudd et al. 1980). Mercury levels in the water of these impoundments were about 40 ng/L and in the lake water about 20-30 ng/L (J. Rudd, personal communication).

DISCUSSION

The small impoundment experiment was easy to implement and the results were similar to those obtained from the larger systems. The only exception was the response of the total rotifer community. The biomass of the rotifers in the small impoundments did not appear to be affected by the presence of 0.1 mg/L Se, but generally decreased in biomass in the large impoundments at this concentration. The only rotifer present in numbers high enough to be converted to biomass in the small impoundments was *K. cochlearis*, whereas in the large impoundments various species were present in such numbers over time, so that the two data sets are not strictly comparable. From 15 July - 9

Table 2. Biomass of zooplankton community and community components in 20 L impoundments, μg/L dry weight ± S.E.

Nominal Selenium Concentration mg/L	a) 0.0 (n=6)	b) 0.1 (n=6)	c) 1.0 (n=6)
Total zooplankton	109.4 ± 10.2	71.7 ± 10.3**	44.0 ± 5.6***
Total calanoid copepods	4.7 ± 0.9	2.1 ± 0.9*	0.0***
Total cyclopoid copepods	45.8 ± 5.2	47.8 ± 4.7	44.0 ± 5.6
Cyclopoid copepod nauplii	3.5 ± 0.4	4.0 ± 0.2	2.7 ± 0.2*
Total cladocerans	57.6 ± 7.9	20.3 ± 6.3***	0.0***
Total rotifers	1.3 ± 0.1	1.4 ± 0.1	0.1 ± 0.02***
Keratella cochlearis	1.1 ± 0.1	1.4 ± 0.1*	0.02 ± 0.01***
Verified selenium concentrations (mg/L)	< 0.001	0.10	1.01

*P = 0.05, **P = 0.01, ***P = 0.001 in comparisons of a) (control) with b) or c).

Table 3. Number/L of zooplankton community and community components in 20 L impoundments ± S.E.

Nominal Selenium Concentration mg/L	a) 0.0 (n=6)	b) 0.1 (n=6)	c) 1.0 (n=6)
Total zooplankton	176.6 ± 10.3	167.1 ± 6.0	97.7 ± 4.4***
Total calanoid copepods	2.2 ± 0.5	1.2 ± 0.4	0.0***
Total cyclopoid copepods	119.1 ± 9.0	125.2 ± 5.9	96.6 ± 4.5*
Cyclopoid copepod nauplii	61.3 ± 5.6	68.9 ± 4.6	42.6 ± 2.8**
Total cladocerans	27.1 ± 2.5	7.8 ± 1.6***	0.0***
Total rotifers	28.2 ± 3.4	33.0 ± 2.4	1.1 ± 0.3***
Keratella cochlearis	25.2 ± 2.9	31.6 ± 2.2	0.5 ± 0.3***
Verified selenium concentrations (mg/L)	< 0.001	0.10	1.01

*P = 0.05, **P = 0.01, ***P = 0.001 in comparisons of a) (control) with b) or c).

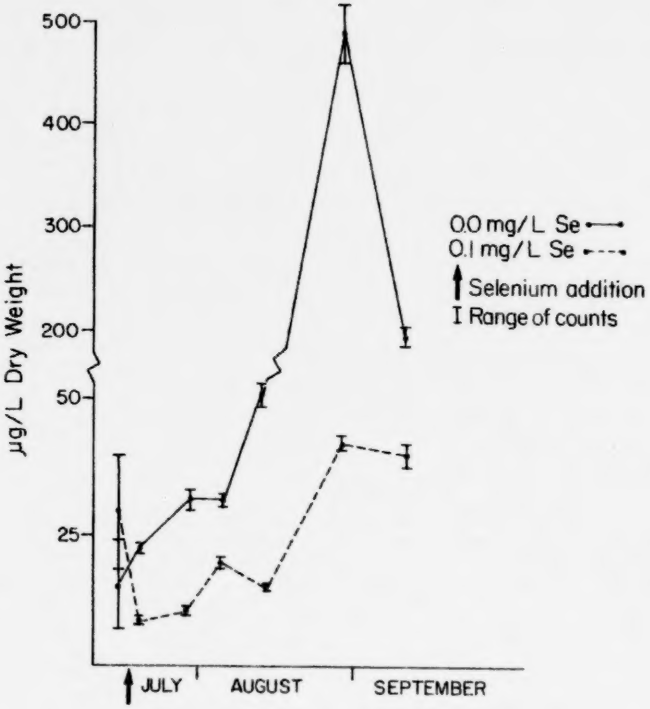

FIG 1. TOTAL ZOOPLANKTON BIOMASS IN LARGE IMPOUNDMENTS, uG/L DRY WEIGHT, 15 JULY - 9 SEPTEMBER.

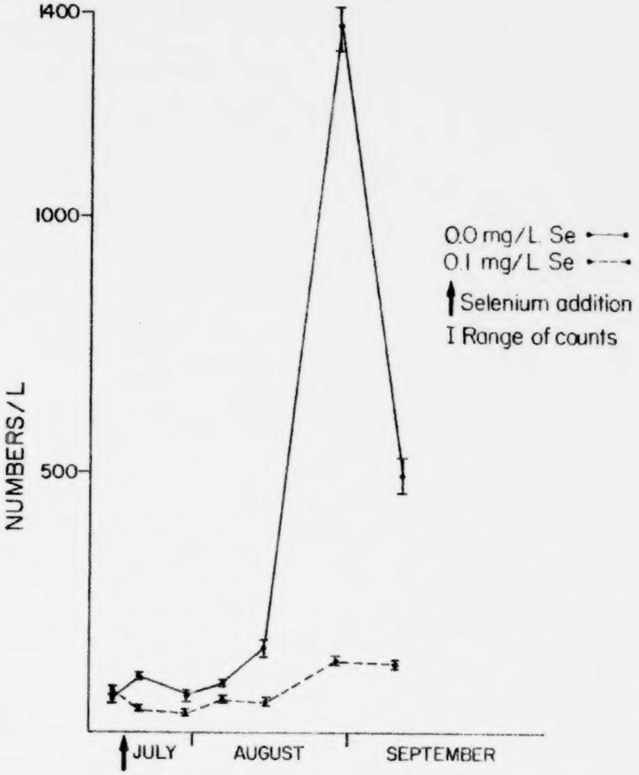

FIG. 2 TOTAL ZOOPLANKTON NUMBERS/L IN LARGE IMPOUNDMENTS, 15 JULY - 9 SEPTEMBER .

Table 4. Total and proportional zooplankton biomass from 15 July - 9 September in large impoundments, μg dry weight/L. Values are averages of seven samples taken over the experimental period.

Nominal Selenium concentration mg/L	0.0	0.1	% Change
Total zooplankton biomass	121.3	24.3	80
Cyclopoid copepods	20.5	19.2	-
Calanoid copepods	7.9	0.7	92
Cladocerans	87.5	3.7	96
Rotifers	5.3	0.6	87

Table 5. Total and proportional zooplankton numbers/L from 15 July - 9 September in large impoundments.

Nominal Selenium Concentration mg/L	0.0	0.1	% Change
Total zooplankton numbers	336.9	78.0	77
Cyclopoid copepods	61.6	63.0	-
Calanoid copepods	9.4	1.6	83
Cladocerans	230.1	1.2	99
Rotifers	35.8	12.2	66

August, the populations of *K. cochlearis* in both control and experimental large impoundments fell steadily from about 170/L to 4-8/L. Rotifer populations are generally larger during spring and fall and reaction to supposed toxic materials could better be examined at those times. *Leptodora kindti*, a large carnivorous cladoceran, apparently did not respond to 0.1 mg/L Se as did herbivorous cladocerans. Since this predator was present in small numbers and not represented in all subsamples counted, these data are highly variable. *L. kindti* was never found in subsamples of impoundments which contained 1.0 mg/L Se.

Total zooplankton biomass was significantly decreased in the presence of 0.1 mg/L Se, by 35% in 10 days in small impoundments and by 80% over the 56 day experimental period in the large. The comparison of data in Tables 2-5 indicates that affected organisms do not acclimate to the presence of selenium over two months.

Change in number as a criterion to assess effects of toxicants on zooplankton is apparently not as sensitive as change in biomass. A comparison of Table 2 and 3 shows that changes in the presence of 0.1 mg/L Se are significant for total biomass and for the biomass of calanoid copepods, cladocerans and *K. cochlearis*. If numbers are considered, the difference is significant only for cladocerans. The use of both biomass and numbers as criteria for assessment is suggested, since retardation of growth and of phases of life cycle can be examined as well as gross change in number.

Should selenium be used for amelioration of the effects of mercury, the concentrations tested are too high for sustained production of the present zooplankton community in Clay Lake. Recent information (J. Rudd, pers. comm.) has suggested that 0.001 mg/L or less may have an ameliorative affect, but toxic effects of such selenium levels to zooplankton are unknown.

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ALGAL FLUOROMETRIC DETERMINATION OF THE POTENTIAL PHYTOTOXICITY OF ENVIRONMENTAL POLLUTANTS

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MOODY, R.P., P. WEINBERGER, and R. GREENHALGH. 1981. Algal fluorometric determination of the potential phytotoxicity of environmental pollutants. Can. Tech. Rep. Fish. Aquat. Sci.

A rapid method for determining the algicidal activity of environmental pollutants was developed. The method relied on the fluorometric determination of algal photosynthetic activity with a miniaturized Kautsky apparatus. Several pesticides and formulation adjuvants used in spruce budworm control spray programs were tested for their ability to inhibit photosynthesis in the green alga, *Chlamydomonas reinhardii*. Concentrations of test chemicals causing 100% inhibition of the fluorescence response (P-T transient) are reported as ICF$_{100}$ values. The most toxic constituents of the Fenitrothion and Matacil formulations were Aerotex 3470 (ICF$_{100}$ of 1-5 ppm) and Nonyl Phenol (ICF$_{100}$ of 0.5 - 0.75 ppm), respectively. The potential use of algal fluorometry as a rapid screening procedure is discussed.

Key words: Phytotoxicity; fluorometry; Kautsky; pesticide; Nonyl Phenol; algae; *Chlamydomonas*.

MOODY, R.P., P. WEINBERGER, and R. GREENHALGH. 1981. Algal fluorometric determination of the potential phytotoxicity of environmental pollutants. Can. Tech. Rep. Fish. Aquat. Sci.

Une méthode rapide pour déterminer le potentiel algicide des polluants environnementaux a été développée. Cette méthode est basée sur la détermination fluorométrique de l'activité photosynthétique des algues à l'aide d'un appareil Kautsky miniaturisé. L'habilité d'inhiber la photosynthèse chez l'algue verte *Chlamydomonas reinhardii* a été mesurée pour plusieurs pesticides et leurs différentes formulations employés pour contrôler la tordeuse de l'épinette. Les valeurs ICF$_{100}$ sont les concentrations des produits chimiques testés qui causent une inhibition totale de la réaction fluorescente (P-T temporaire). Les composantes les plus toxiques des formulations de Fénitrothion et de Matacil sont respectivement Aerotex 3470 (ICF$_{100}$ de 1-5 ppm) et Nonyl Phenol (ICF$_{100}$ de 0.5 - 0.75 ppm). Le potentiel de cette méthode de fluorométrie des algues pour effectuer des tests rapides est discuté.

INTRODUCTION

Fluorescence induction determinations are sensitive indicators of photosynthetic activity (for review, cf. Papageorgiou 1975) and have been used to determine the effects of environmental conditions (temperature, CO_2, O_2, light, etc.) on algal photosynthesis (Krause 1973; Schreiber and Vidaver 1974) and ozone on photosynthesis in higher plants (Schreiber et al. 1978). The present study describes a rapid fluorometric screening procedure designed to determine the relative algicidal activity of pollutants (pesticides, petroleum hydrocarbons, industrial effluents, etc.) that contaminate aquatic habitats. The effects of pesticides and petroleum hydrocarbons on algae have been reviewed by Butler (1977), and O'Brien and Dixon (1978), respectively.

Since reporting that fenitrothion accumulated in aquatic plants following spruce budworm spray programs (Moody et al. 1978), concern was expressed that the insecticide or its formulation adjuvants (Aerotex 3470 and Atlox 3409F) might be phytotoxic to unicellular phytoplankton. Subsequent studies have demonstrated several toxic effects of the petroleum distillate, Aerotex 3470, on the green alga, *Chlamydomonas reinhardii* (Moody et al. 1981). The present study reports the use of a miniaturized Kautsky apparatus (Schreiber et al. 1975) for determining the % inhibition of photosynthesis of *C. reinhardii* treated with two insecticides (an organophosphate, Fenitrothion and a carbamate, Matacil) and several formulation adjuvants (Aerotex 3470, Atlox 3409F, Dowanol, Diluent-585 and Nonyl Phenol) that have been used extensively in budworm spray programs (Symons 1977; Varty 1980).

MATERIALS AND METHODS

Algal Culture

Chlamydomonas reinhardii (+ strain) was obtained from the Culture Collection at Indiana University and was cultured in autoclaved Bold's Basal Medium (BBM), pH 6.7 (Bold 1949). Algal cultures (1 L) were held in 2,800 mL Fernbach type flasks (pyrex No. 4420) on an Eberach shaker (78 oscillations per minute). Cultures were maintained in a Hotpack growth chamber (5 klx, 16 h light: 8 h dark photoperiod, 23°C). Cell counts were made with an improved Neubauer hemacytometer.

Algal Treatment

The pesticides and adjuvants selected for screening and their source of origin are listed in Table 1. Stock preparations of each test compound (100 mg/100 mL) were made immediately prior to use by one of two methods:

1. Stock solutions of non-volatile, acetone-soluble compounds were prepared with acetone. Suitable aliquots of stock solution were dispensed into 50 mL screw-cap Erlenmeyer flasks. The acetone was blown off under an air stream and 45 mL of BBM containing sufficient $NaHCO_3$ (CO_2 source) to provide a 1 mM concentration in 50 mL were immediately added to each flask.

TABLE I

Test Chemical, Description and Source

Chemical	Description	Source
Fenitrothion	O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate	Sumitomo Chemical Co.
Aerotex 3470	petroleum distillate	Texaco Canada Ltd.
Atlox 3409F	detergent mixture	Atlas Chemical Co.
Dowanol	dipropylene glycol methyl ether	Dow Chemical Co.
Matacil	4-dimethylamino-m-tolyl methyl carbamate	Chemagro Ltd.
Matacil 1.8-D[1]	commercially prepared field formulation containing Matacil, Nonyl Phenol and Diluent-585 (1.00:2.54:1.52 (v/v))	Chemagro Ltd.
Nonyl Phenol	mixture of monoalkyl phenols (predominantly para substituted)	Chemagro Ltd.
Diluent-585	petroleum distillate	Chemagro Ltd.

[1] Final treatment preparation followed the field dilution recipe provided by Chemagro Ltd. (Matacil 1.8-D: Diluent-585 (1:1.88)).

An identical aliquot (5.0 mL) of a 9 - 12 day old culture of *Chlamydomonas* in late log phase was then added to each flask to provide a final cell concentration of 2.6×10^4 cells/mL. The flasks were capped tightly, shaken and placed on a shaker in the dark at 23°C. Dark conditions were used to negate the possibility of photodegradation of the toxicant.

2. Water soluble or volatile test compounds were suspended in BBM by mixing in a Polytron sonicator (No. 4369) at setting No. 5. Subsequent treatment followed the procedures outlined above for the acetone stock solutions, except that the total volume was corrected for the addition of algal medium.

A wide range of treatment concentrations (0.1, 1, 10 and 100 ppm) was initially tested to determine the proper range for subsequent studies. Triplicate flasks were used for each treatment concentration.

Fluorometric Analysis

A Plant Productivity Fluorometer (model SF-10) was obtained from Richard Brancker Research Ltd., Ottawa, Canada. The fluorometer was used in conjunction with a Fisher Recordall® Series 5000 chart recorder (signal input 10 vdc, chart speed 13 cm/min). The fluorometer was set at a light (670 nm) exposure of 10 sec and at maximum intensity (10^4 ergs/cm^2/sec).

After a 1-h treatment each 50 mL algal sample was filtered under vacuum through a Whatman GF/C glass fibre filter. The glass filter was then placed on top of two Whatman No. 1 filter papers pre-moistened with BBM and the fluorometer probe was centered on top. During the filtration step and positioning of the probe, the algae were inevitably exposed to light. This reduced the sensitivity of the fluorometer response and it was necessary to readapt the cells to the dark. This was accomplished by leaving the probe on top of the algae for 30 sec. An exact time period was required to obtained reproducible results. In this connection, previous studies have shown that the size of the initial fluorescence peak is directly proportional to the length of the preceding dark period (Krause 1972). A period of 30 sec dark adaptation was found to give sufficient sensitivity of response to permit accurate quantitation of the fluorescence transients in the present study. Duplicate analyses were made for each sample by repositioning the probe on the glass filter. Finally, the glass filter was washed under vacuum with 25 mL of BBM and re-analyzed to determine the reversibility of the treatment effect.

Following the above procedure, duplicate analyses of triplicate samples could be performed in less than 10 min. A maximum of 2 h was required to determine the minimum concentration required for any compound to totally suppress the fluorescence response.

RESULTS

Figure 1 shows a typical fluorescence transient obtained for untreated (control) cultures of *Chlamydomonas* showing the peak (P) characteristic of photosynthetically active cells which was absent for treated cultures. O, P, M and S divide the fluorescence transient into segments that are indicative of several photosynthetic partial reactions (Papageorgiou 1975). The letter "T" on Figure 1 designates the termination of peak "P". The length of the P-T transient was used to quantitate photosynthetic activity.

A progressive decrease in the length of the P-T transient was observed as the treatment concentration of a test compound neared the level at which the fluorescence transient became flat as in Figure 1. A flat fluorescence transient was also obtained for heat-treated (80°C), frozen (-60°C) or desiccated algae in the present study, and is generally held to be indicative of the absence of photosynthetic activity (Papageorgiou 1975). The results obtained were quantitated by measuring the length of the P-T transient and expressing this as a percentage reduction (% inhibition, Table 2) of the control value. An analysis of variance was performed initially on the 6 replicate P-T values (3 replicate flasks x 2 sample analyses) obtained for each treatment concentration. In all cases, $F_{2,3}$ was less than 9.55 ($P < 0.95$), permitting reporting data as a pooled mean ± standard deviation (Table 2).

The data obtained for the constituents of the Fenitrothion and Matacil formulations are given in Table 2. When a pesticide formulation was used, the fluorometer results were reported for the concentration present for one of the formulation constituents. For example, the fluorometer data recorded for Matacil 1.8 D (Matacil: Nonyl Phenol: Diluent-585 [1.00:2.54:1.52 (v/v)] was reported for the concentration of Nonyl Phenol present during treatment (Table 2, footnote 3).

The second column (TR) in Table 2 indicates the treatment procedure used. As discussed previously, the test compound was applied either in acetone which was then volatilized, or solubilized directly in BBM. It is imperative to note the importance of adopting a correct treatment procedure. For example, Aerotex at 10 ppm did not inhibit the fluorometer response in *Chlamydomonas* if Aerotex was dissolved in acetone and then exposed to an air stream for 5 min prior to treatment. If applied in aqueous emulsion, however, Aerotex at 5 ppm was sufficient to totally suppress the fluorometer (P-T transient) response (Table 2). In this connection gas chromatographic (GC) analysis (Moody et al. 1981) of Aerotex standards prepared in acetone demonstrated that $\geq$ 50% of each of the hydrocarbon constituents of Aerotex had volatilized after 10 min exposure to an air stream. Similar GC analyses of Fenitrothion and Matacil standards did not demonstrate significant volatilization after 10 min exposure to an air stream. A maximum of only 15 - 20 sec exposure to the air stream was sufficient to volatilize the acetone in the toxicity studies.

Generally, the results (Table 2) demonstrated a slight increase in the length of the P-T transient at low concentrations of the test compounds. Higher treatment concentrations were associated with a decrease in the length of the P-T transient which was reported as an increase in the % inhibition of

FIGURE 1

The fluorescence transient in Chlamydomonas reinhardii: 670 nm; incident intensity 10^4 ergs/cm^2/sec. The solid line depicts a typical transient for untreated cells showing the points O, P, T, M and S. The hatched line depicts a typical transient for cultures inhibited by chemical treatment.

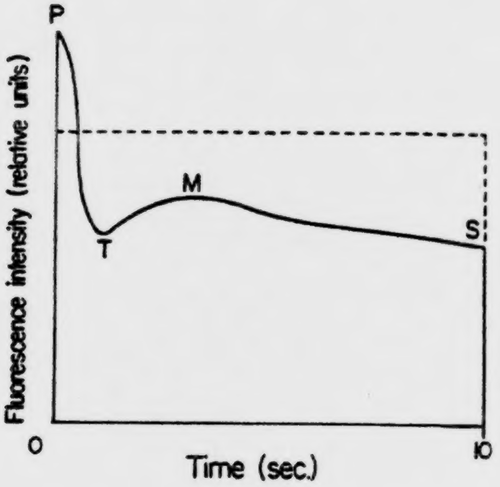

TABLE 2

Inhibition (%) of photosynthesis as determined by fluorometric analysis of _C. reinhardii_ treated for 1 hour.

Chemical	TR[1]	Treatment Concentration (ppm)											
		0	0.1	0.2	0.5	0.75	1.0	5.0	10	20	30	40	100
Fenitrothion	D	0 (13.6)					-11.4[4] (31.1)	40.9 (19.7)	68.2 (7.6)	100 (0)			
Dowanol	W	O (5.1)					-3.2 (10.2)	-1.9 (7.0)	6.4 (12.7)				2.5 (21.7)
Atlox	W	0 (13.6)					-11.7 (17.4)	-1.9 (13.2)	5.2 (11.7)	60.1 (19.3)	100 (0)	100 (0)	
Aerotex	W	0 (6.1)					52.8 (9.8)	100 (0)	100 (0)	100 (0)			
Fenitrothion:Aerotex:Atlox[2] (10:1:1)	W	0 (4.8)			-16.8 (11.1)		-9.6 (10.6)	25.5 (13.9)	41.3 (9.1)	100 (0)			
Matacil	D	0 (14.9)					6.9 (4.6)	10.3 (9.1)	10.3 (5.7)	15.4 (4.6)			51.4 (8.0)
Diluent-585	W	0 (14.7)					-8.0 (10.7)	8.0 (8.0)	3.3 (4.0)	71.3 (5.3)	100 (0)	100 (0)	100 (0)
Nonyl Phenol	D	0 (7.8)	-17.2 (14.1)	0 (11.7)	54.7 (16.4)	100 (0)	100 (0)						
Matacil 1.8-D[3]	D	0 (16.8)	-3.2 (16.1)		80.6 (12.3)	100 (0)	100 (0)						

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1 D = Dry treatment (in acetone blown off); W = Wet treatment (in BBM)

2 Results are reported for the Fenitrothion concentration (ppm) of the formulation

3 Results are reported for the Nonyl Phenol concentration (ppm) of formulation

4 A negative value was obtained when the P-T transient was greater than the control

5 Standard deviation of 6 replicate analyses is given in brackets

photosynthesis. The length of the P-T transients before and after washing with BBM did not differ significantly, except for Matacil at 100 ppm, which before washing gave 51.4 ± 8.0% inhibition as compared to 21.7 ± 6.6% after washing.

The lowest concentration of a test compound required to totally suppress the fluorometer response (P-T = 0) will be designated here as the ICF_{100} value. This value is reported as a concentration range for each test compound in Table 3. For example, 1 ppm of Aerotex was shown to cause 52.8% inhibition of photosynthesis while 100% inhibition was recorded for 5 ppm (Table 2). Hence, the ICF_{100} value for Aerotex is reported as 1 - 5 ppm (Table 3). The ICF_{100} value for Fenitrothion (10 - 20 ppm) was the same for the pesticide, either alone, or in formulation with Aerotex and Atlox, or Dowanol and Atlox. Similarly, the ICF_{100} value for Nonyl Phenol was from 0.5 - 0.75 ppm, either alone or when present in the Matacil 1.8-D formulation. The ICF_{100} values reported in Table 3 were obtained for a 1 hr treatment duration. Figure 2 plots the % inhibition of photosynthesis (decrease of P-T transient) versus duration of treatment with 5 ppm (upper limit of ICF_{100}, Table 3) of Aerotex. The time required to cause 50% inhibition was less than 10 minutes, while flat transients were obtained 30 min post-treatment. Flat transients were also obtained after 24 h treatment for all test chemicals when treatments were conducted at the upper range of the respective ICF_{100} value. The 24 h control algae still exhibited the P-T transient.

DISCUSSION

The present study was undertaken to establish a rapid reproducible means for assessing the relative algicidal activity of a wide array of environmental pollutants. The results indicate that algal fluorometry could provide a suitable screening procedure for further laboratory and field studies. The method is extremely rapid (maximum of 2 h required to determine ICF_{100} for any compound), the apparatus is portable for field use, and sensitivity would depend only on the algal species selected for screening. A wide variety of species could be employed in future studies to establish an order of species tolerance that could be used to relate the species composition of endemic algal populations to the water quality of a particular habitat. Such a table has been composed previously (Palmer 1969) by arbitrarily conferring extra tolerance to those algal species reported most frequently inhabiting polluted water. It is notable that C. *reinhardii* was among the most tolerant species listed.

The observation that 1 - 5 ppm of Aerotex was sufficient to totally suppress the fluorometer response (ICF_{100} = 1 - 5 ppm) in C. *reinhardii* was consistent with our previous report that Aerotex (1 - 10 ppm) inhibited cell motility, population growth, ATP synthesis and induced ultrastructural changes in membrane configuration (Moody et al. 1981). The enhanced photosynthesis suggested by the fluorometer results (Table 2) for low concentrations of several test chemicals was consistent with enhanced $^{14}CO_2$ fixation demonstrated in our laboratory for low Aerotex concentrations. Further, the reported ICF_{100} values were consistent with the results of extensive tests conducted in our laboratory with standard bioassay procedures (population growth, $^{14}CO_2$

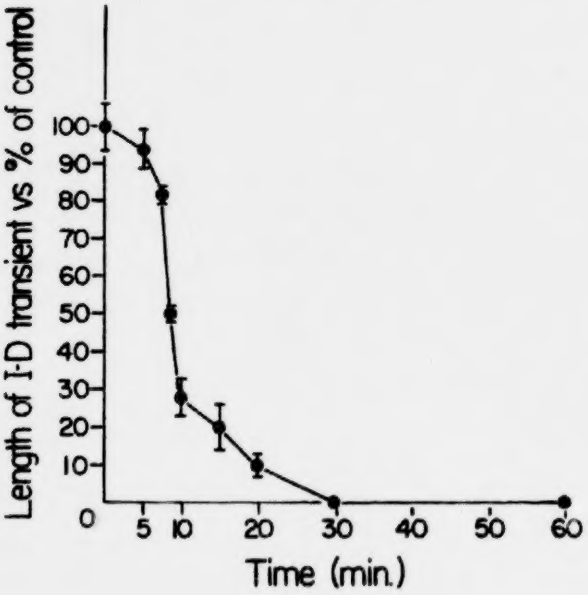
TABLE 3

ICF_{100} values - concentration (mg/l) required to totally inhibit photosynthesis in Chlamydomonas reinhardii within 1 hr of treatment.

Chemical	ICF_{100} (ppm)
Fenitrothion	10-20
Aerotex 3470	1-5
Atlox 3409F	20-30
Dowanol	over 100
Fenitrothion:Aerotex:Atlox (10:1:1)	10-20 (Fenitrothion)
Fenitrothion:Dowanol:Atlox (10:1:1)	10-20 (Fenitrothion)
Matacil	over 100
Matacil 1.8-D	0.5-0.75 (Nonyl Phenol)
Nonyl Phenol	0.5-0.75
Diluent-585	20-30

FIGURE 2

Percent inhibition of fluorometer (P-T) transient versus duration of treatment with Aerotex (5 ppm). Mean values of triplicate samples are shown together with standard deviation bars.



fixation, cell leakage studies, etc.) (Moody 1982).

The relevance of the present results concerning the *in situ* algicidal potential of Aerotex has been discussed previously (Moody et al. 1981). Of even greater consequence was the observation that the ICF_{100} of Nonyl Phenol for C. *reinhardii* was between 0.5 and 0.75 ppm, either alone, or when present in the Matacil 1.8-D field formulation recently sprayed in New Brunswick, where levels as high as 0.1 ppm of Nonyl Phenol have been reported in standing water following experimental applications (albeit under "worst case" conditions) (Caldwell *in* Varty 1980). Holmes and Kingsbury (1980), however, reported levels of Nonyl Phenol up to 1.1 ppm in stagnant water following an operational spray program in Ontario. When assessing field reports of pesticide concentrations in water, it is essential to consider the effect of dilution within the water column. Initial concentrations in the surface layer would be relatively high, and the present results indicate that even a brief (1 h) exposure of algae inhabiting this layer (e.g., neuston) would suffice to inhibit *in situ* photosynthesis.

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BIOACCUMULATION OF HEAVY METALS BY SCULPIN (*COTTUS ASPER*) EXPOSED TO MINE TAILINGS

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REID, B.J. and E.R. MCGREER. 1981. Bioaccumulation of heavy metals by sculpin (*Cottus asper*) exposed to mine tailings. Can. Tech. Rep. Fish. Aquat. Sci.

Twenty-eight day laboratory tests examined the uptake of Cu, Pb, Zn, and Cd in muscle tissue of the freshwater sculpin *Cottus asper* exposed to mine tailings discharged into Buttle Lake, B.C. Sculpin tissue was analyzed at the beginning of the experiment and after 4, 14 and 28 days. Water samples were drawn off at each sampling occasion to assess the rate of metal leaching, and its relationship to bioavailability. Results showed a steady increase in levels of dissolved Cd, Pb and Zn, but fluctuating levels of Cu in water overlying 100% tailings and a 50:50 sediment/tailings mixture throughout the 28 day period. After 28 days, Cu and Pb levels in sculpins exposed to 100% tailings and the 50:50 mixture were higher than initial background levels. Elevated tissue concentrations for Zn were recorded in sculpins exposed to 100% tailings. Of the metals bioaccumulated, only zinc levels exceeded those found in controls. Resident lake biota (sculpins and plankton) were also collected to determine the degree of metal accumulation in natural populations. Levels of all metals were generally higher in resident biota from lakes downstream of the mine discharge, than from a nearby lake unaffected by the mine tailings. Extremely high metal levels were recorded in lake plankton which appeared to be better indicators of metal bioavailability than the fish species sampled.

Key words: Bioaccumulation, heavy metals, mine tailings, fish.

REID, B.J. and E.R. MCGREER. 1981. Bioaccumulation of heavy metals by sculpin (*Cottus asper*) exposed to mine tailings. Can. Tech. Rep. Fish. Aquat. Sci.

L'absorption de Cu, Pb, Zn et Cd par le tissu des muscles du chabot piquant (*Cottus asper*) exposé aux déchets de mine déchargés dans le lac Buttle, C.B. a été mesurée par des tests de laboratoire d'une durée de 28 jours. Le tissu du chabot piquant est analysé au début de l'expérience et après 4, 14 et 28 jours. Des échantillons d'eau sont ramassés à chaque période d'échantillonnage pour évaluer le lessivage des métaux et la relation entre celui-ci et leur disponibilité aux tissus vivants. Les résultats montrent une augmentation constante dans les niveaux de Cd, Pb et Zn dissous mais des niveaux variables de Cu dans l'eau des déchets de 100% et d'un mélange 50:50 sédiments:dechets pendant toute la période de 28 jours. Après 28 jours, les niveaux de Cu et Pb dans les chabots piquants exposés aux déchets de 100% et au mélange 50:50 sont plus élevés que les niveaux initiaux de base. Des concentrations de Zn élevées sont enregistrées chez les chabots piquants exposés aux déchets de 100%. Des métaux

accumulés dans les tissus, seuls les niveaux de zinc excèdent ceux trouvés dans les contrôles. Le biote lacustre (chabots et plancton) est aussi échantillonné pour déterminer le niveau d'accumulation des métaux dans les populations naturelles. Les niveaux de tous les métaux sont généralement plus élevés dans le biote des lacs en aval de la décharge des mines que dans un lac voisin non-affecté par ces déchets. Des niveaux de métaux extrêmement élevés sont enregistrés dans le plancton lacustre. Le plancton semble être un meilleur indicateur de disponibilité de métaux pour les tissus vivants que l'espèce de poisson étudiée pour certains crustacés se nourrissant par filtration.

La réduction dans le niveau d'oxygène des enclos traités à l'atrazine peut avoir stressé tout la communauté de zooplancton.

INTRODUCTION

Tailings from a large copper/lead/zinc mine (Westmin Resources) have been discharged for over 15 years into the southern end of Buttle Lake, B.C. The tailings, which contain substantial concentrations of copper, zinc, lead and cadmium are discharged via a submerged pipeline at approximately 26 m depth (Duncan 1974). Concern was expressed by the B.C. Provincial Ministry of Environment over possible deleterious effects of these tailings on salmonid fisheries in Buttle and surrounding lakes. This study was initiated to determine the bioaccumulation of heavy metals from mine tailings in a representative freshwater salmonid food organism (sculpins) in laboratory tests, and to relate these results to metal levels found in resident biota.

METHODOLOGY

FIELD COLLECTIONS

Fish

Sculpins (*Cottus asper*) for heavy metal analyses were collected from Buttle, Upper Campbell, Campbell, John Hart and Upper Quinsam Lakes (Fig. 1) using baited crab or minnow traps. Whole fish were placed in individual WHIRLPAK bags and immediately frozen on site by placing in a cooler with ice.

Sculpins (*Cottus asper*) for bioaccumulation tests were collected using the methods described above from a lake with no known pollution input (Stave Lake) near Vancouver, B.C. Sculpins were transported to the laboratory in acid washed, distilled water rinsed plastic containers and placed in flow-through freshwater holding tanks (12 ± 1°C) for one week acclimation before testing.

Plankton

Plankton for heavy metal analyses were collected from Buttle, Upper

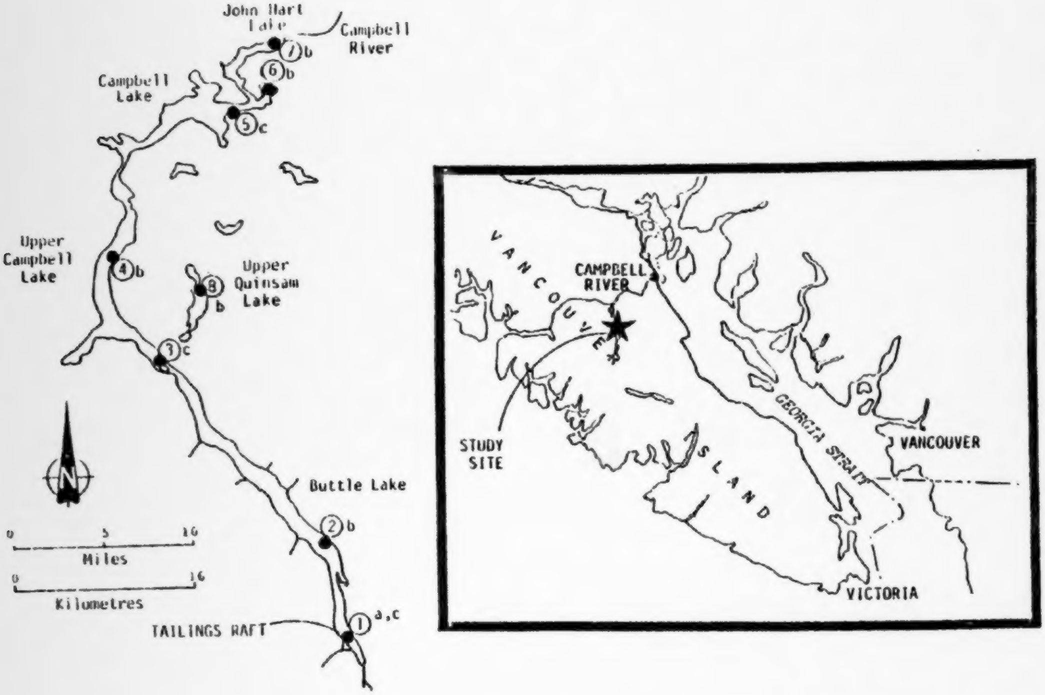
John Hart Lake
Campbell River
Campbell Lake
7 b
6 b
5 c
Upper Campbell Lake
4 b
Upper Quinsam Lake
8 b
3 c
N
Buttle Lake
2 b
0
5
10
Miles
0
16
Kilometres
TAILINGS RAFT
1 a,c
VANCOUVER ISLAND
CAMPBELL RIVER
STUDY SITE
GEORGIA STRAIT
VANCOUVER
VICTORIA

Figure 1. Study site and sampling locations for mine tailings and resident biota.

a = mine tailings

b = sculpin

c = plankton

Campbell and Campbell Lakes using a 1 m wide, 63 μm mesh plankton net towed behind a boat approximately 1 - 1.5 m below the water surface. Plankton were placed in individual WHIRLPAK bags and frozen on site.

Sediment

Sediments were collected from various locations in Buttle Lake using a Ponar Grab sampler, and examined on site for presence of benthos which could be collected for metal analysis. Benthic animals were present at all sites but there was insufficient biomass for chemical analysis. Sediments used in controls in bioaccumulation tests were obtained from Rolly Lake, near Vancouver, B.C. Samples (200 g) were retained for analysis of metals, total organic carbon and particle size distribution. Remaining sediments were frozen prior to use in experiments to reduce bacterial contamination.

Mine Tailings

Mine tailings were collected from inside the Westmin Resources discharge pipe located on the tailings raft at the southern end of Buttle Lake. Samples of the solid phase of the tailings were placed in individual WHIRLPAK bags for later analysis of particle size distribution, metals, and total organic carbon. Remaining tailings were held at 4^{o}C.

LABORATORY METHODS

Bioaccumulation Studies

Twenty-eight day static exposure tests were conducted in 77 L polyethylene containers at 10 ± 0.5^{o}C under a 12 h light/dark cycle. A plastic undergravel filter consisting of a 1 cm grid (26 x 26 x 3 cm) covered with 2 mm plastic mesh, and a vertical stack (25 x 2.5 cm) was placed on the bottom of each container (Fig. 2). Air passing through an airstone in the stack promoted water circulation through the tailings.

Three different tailings/sediment conditions were used to assess bioaccumulation potential: 100% tailings, a 50:50 mixture of mine tailings and clean sediment, and a clean sediment for control. The 100% tailings consisted of 45 L of tailings as obtained from the outfall (a slurry of solid and liquid phases). The 50:50 mixture was prepared by combining 3.5 L each of solid tailings phase and clean sediments in the bioaccumulation chamber which was then covered with 18 L each of liquid tailings phase and dechlorinated city freshwater. The control consisted of 7 L of clean sediment with 38 L of dechlorinated freshwater. Sediments were left to settle for 24 h prior to starting the test. All test apparatus was pre-rinsed with dilute nitric acid, then twice rinsed with distilled water.

Prior to introduction of test organisms, dissolved oxygen levels were raised to 90% saturation and water pH was adjusted to 7.0 ± 0.2. Six sculpins (*Cottus asper*) of approximate equal length (83 mm average) were placed in each

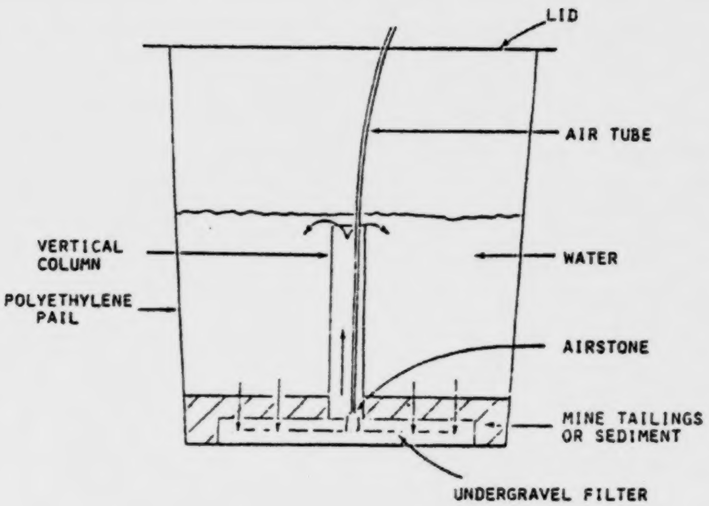
LID
AIR TUBE
VERTICAL COLUMN
WATER
POLYETHYLENE PAIL
AIRSTONE
MINE TAILINGS OR SEDIMENT
UNDERGRAVEL FILTER

Figure 2. Experimental set-up for bioaccumulation studies.

test chamber such that loading densities were less than 1.0 g/L. Sculpins were left unfed for the test duration. Dissolved oxygen levels, pH and conductivity were measured daily throughout the test. Fish mortalities and sublethal effects were also noted during these daily observations.

Water samples (300 mL) for metal analysis were drawn off each container 5 cm above the sediment surface with a pre-rinsed glass pipette prior to introducing the fish, and after 4, 14 and 28 days. On each occasion two sculpins were removed and immediately frozen whole in individual WHIRLPAK bags for later tissue metal analysis.

Analytical Methodology

Water samples were filtered (0.45 μm millipore) immediately after sampling, then analyzed for dissolved lead and cadmium by graphite furnace atomic absorption, and copper and zinc by direct flame atomic absorption.

Muscle tissue was dissected from frozen sculpins using stainless steel instruments rinsed between samples with dilute HNO_3 and distilled water. The tissue was rinsed twice in distilled water then frozen until analysis. Plankton samples were filtered, and tissue recovered for analysis. Samples were dried to determine moisture content then digested using a combination of nitric and perchloric acids. All resulting solutions were analysed by direct AAS as described above.

Sediments and tailings were filtered to recover solids, dried and digested using a combination of nitric and perchloric acids and analysed as described above. Total organic carbon was determined by Leco Induction Furnace. Particle size distribution was determined by wet sieving down to 63 μm then using the pipette method to obtain size fractions to < 4 μm.

To ensure quality control, blanks were run for each element while certified reference standards were digested and analysed concurrently with each batch of samples.

RESULTS AND DISCUSSION

Data on metal concentrations, particle size distribution and total organic carbon levels in sediments and tailings are listed in Table 1. Levels of all metals were higher in tailings compared to control sediments, with the 50:50 tailings:sediment mixture having intermediate concentrations. Control sediments consisted of slightly larger particles compared to tailings, while total organic carbon levels were identical (0.14%) in both tailings and control sediment. Conductivity, pH and dissolved oxygen levels fluctuated slightly within each chamber (Table 2).

Metal Leaching

Metal levels in water overlying sediment and tailings during the 28 day

TABLE 1

INITIAL SEDIMENT AND MINE TAILINGS CHARACTERISTICS

SEDIMENT	TOTAL ORGANIC CARBON (%)	PARTICLE SIZE DISTRIBUTION				METAL LEVELS (μg/g dry weight)			
		>2 mm	<2 mm to 63 μ	63 μ to 4 μ	4<4 μ	Cu	Pb	Zn	Cd
Initial Clean Sediment	0.14	11.2%	86.6%	1.2%	1.0%	10.	<2.5	18.5	<0.10
50/50 Sediment Tailings	-	-	-	-	-	463.	385.	2920.	11.5
Mine Tailings	0.14	-	32.4%	48.5%	19.1%	820.	665.	5250.	22.0

TABLE 2

PHYSICAL DATA FOR BIOACCUMULATION TESTS

	CONTROL			SEDIMENT/TAILINGS MIX			100% TAILINGS		
TIME (DAY)	pH	D.O. (mg/L)	CONDUCTIVITY (μohms/cm)	pH	D.O. (mg/L)	CONDUCTIVITY (μohms/cm)	pH	D.O. (mg/L)	CONDUCTIVITY (μohms/cm)
0	7.0	11.2	30	7.0	11.7	700	7.0	11.6	1200
1	5.8	11.7	22	7.1	11.1	650	7.5	11.5	1220
2	6.4	11.3	25	7.3	11.7	660	7.3	11.8	1210
3	6.1	11.6	20	7.6	11.5	650	7.5	11.3	1210
*4	6.2	11.8	28	7.3	11.2	650	7.4	11.3	1210
5	6.0	11.6	19	7.3	11.4	650	7.6	11.5	1210
6	6.0	11.3	20	7.4	10.7	620	7.6	11.3	1195
7	6.0	11.4	20	7.4	10.8	630	7.6	11.4	1210
8	5.8	11.5	22	7.6	10.9	660	7.5	11.5	1200
9	6.1	11.6	21	7.6	10.8	625	7.6	11.3	1180
10	6.4	11.5	20	7.6	10.8	630	7.6	11.2	1200
11	7.0	11.5	22	7.7	10.9	660	7.5	11.0	1220
12	6.3	11.4	20	7.4	11.3	650	7.5	11.2	1220
13	6.5	11.6	25	7.6	11.6	650	7.4	11.4	1200
*14	6.3	11.4	23	7.6	11.4	650	7.4	11.2	1200
15	6.3	11.4	23	7.5	11.5	670	7.4	11.5	1210
16	6.2	11.3	23	7.5	11.6	670	7.3	11.4	1220
17	6.2	11.5	22	7.4	11.3	680	7.4	11.4	1220
18	6.0	11.2	25	7.3	11.3	700	7.3	11.5	1220
19	5.9	11.6	25	7.5	11.5	690	7.3	11.3	1210
20	6.4	11.3	22	7.3	11.5	690	7.2	11.2	1200
21	6.2	10.9	25	7.6	11.1	690	7.3	11.1	1220
22	6.6	11.8	22	7.2	12.0	700	7.0	12.0	1250
23	6.4	11.7	23	7.3	11.7	700	7.2	11.3	1220
24	5.9	11.9	25	7.3	12.1	700	7.2	12.0	1200
25	5.8	11.8	25	7.3	11.8	680	7.2	11.9	1200
26	5.8	11.7	23	7.4	11.6	690	7.3	11.9	1210
27	5.8	11.7	23	7.4	11.5	680	7.3	11.4	1200
*28	5.7	11.2	22	7.3	11.4	650	7.3	11.4	1190

*Fish and water sampled for metal levels bioaccumulation tests are shown in Table 3, and illustrated in Figure 3. Lead, cadmium and zinc concentrations increased in water overlying 100% tailings, and in the 50:50 tailings mixture after 28 days (Fig. 3). Copper concentrations fluctuated with levels declining from a peak in all containers after 14 days. Cadmium in the control was below detection limits on all sampling occasions. Lead levels in the control showed a very high peak (10 ppb) at 14 days, but no explanation for this phenomenon was apparent. Zinc leached to the greatest degree over the first 4 days from 100% tailings (690 µg/L to 990 µg/L) but showed a slower rate of increase over the next 24 days (990 - 1440 µg/L).

The leaching and eventual bioavailability of metals from mine tailings is dependent on a number of interacting characteristics of the tailings and environment. Mine tailings are complex mixtures, containing many different matrices and species of heavy metals, depending on the ore and methods of processing (Clarke 1974). Also, environmental factors such as pH, redox potential, and complexing with organic or inorganic particulates will determine the release of metals from tailings (Jenne and Luoma 1977; Burton 1979). Jackson et al. (1980) found that in soft water lakes, the most important trace metal binding agents in water or sediment were biological organisms such as plankton, humic matter and hydrated oxides of iron and manganese.

McGreer et al. (1980) found that leaching of metals from three different mine tailings in freshwater was variable and depended on the tailings studied. Zinc leached most readily over a 30-day period (McGreer et al. 1980) to levels comparable to that of present study. Delisle et al. (1975) found that concentrations of Cu, Pb, Zn and Cd in contaminated sediments declined by 50% when exposed to freshwater for 5 months, with the fine sediment fraction containing the highest residual and initial concentration of metals. In the present study, combining sediment with tailings did not reduce the rate of leaching which suggests binding of metals did not occur onto control sediment particulates. This result is consistent with the low organic carbon content (0.14%) in both the sediment and tailings. Investigations into the species and phases of metals present in the mine tailings would provide useful information in explaining the observed leaching potential.

Laboratory Bioaccumulation

Metals bioaccumulated in sculpin (*Cottus asper*) muscle tissue from laboratory tests are shown in Table 4, and illustrated in Figure 4. No fish mortalities or obvious sublethal effects were observed in any tanks for the 28-day exposure period.

Cadmium in tissues fluctuated slightly in all tanks, but was undetectable after 28 days in sculpins exposed to 100% tailings and 50:50 mixture (Table 4). After 28 days, zinc, lead and copper levels were elevated above initial background levels in sculpins exposed to 100% tailings, however only with zinc were those levels above that of controls. Increases in lead levels were most pronounced in the 50:50 mixture after 28 days while zinc levels were lower than background (49.2 µg/g compared to 37.3 µg/g).

Highest copper, lead and zinc levels were obtained after 4 days exposure to 100% tailings (Table 4). Comparing the two test conditions containing mine

TABLE 3

TOTAL DISSOLVED METAL CONCENTRATIONS (μg/L)
IN FRESHWATER DURING 28-DAY BIOACCUMULATION TESTS

TEST	TIME (DAYS)	METALS (μg/L) Cu	Pb	Zn	Cd
CONTROL	0	2.0	5.0	11.0	<1.0
	4	3.0	1.0	150.0	<1.0
	14	15.0	11.0	27.0	<1.0
	28	2.0	1.0	7.0	<1.0
SEDIMENT/ TAILINGS 50/50	0	28.0	3.0	220.0	1.0
	4	11.0	2.0	480.0	5.0
	14	26.0	10.0	590.0	7.0
	28	21.0	6.0	670.0	5.0
100% TAILINGS	0	82.0	8.0	690.0	3.0
	4	14.0	2.0	990.0	12.0
	14	61.0	11.0	1120.0	16.0
	28	34.0	15.0	1440.0	17.0

TABLE 4

METAL CONCENTRATION (μg/g) IN SCULPIN MUSCLE TISSUE
DURING 28-DAY BIOACCUMULATION TEST

TEST CONDITIONS	TIME (DAYS)	WET TISSUE WEIGHT* (gm)	MEAN FISH LENGTH* (mm)	% MOISTURE	METAL CONCENTRATION* (μg/g dry weight) Cu	Pb	Zn	Cd
INITIAL	0	4.67	118.5	78.7	1.92	0.84	49.2	<0.05
CONTROL	4	1.90	82.5	77.9	3.00	0.86	42.9	<0.05
	14	2.69	87.5	78.8	2.19	0.78	40.2	0.10
	28	1.50	74.0	79.4	2.97	3.65	43.3	0.17
50/50 TAILINGS/ SEDIMENT	4	2.70	83.5	77.4	2.93	2.93	37.7	<0.05
	14	2.05	78.0	82.5	3.09	0.68	50.2	0.10
	28	4.08	93.5	80.8	2.65	4.75	37.3	<0.05
100% TAILINGS	4	1.18	70.0	79.7	5.07	7.41	61.8	0.12
	14	3.15	81.5	81.6	1.00	0.71	46.6	0.06
	28	2.70	91.0	83.3	2.22	2.47	54.9	<0.05

*Based on composite of two fish

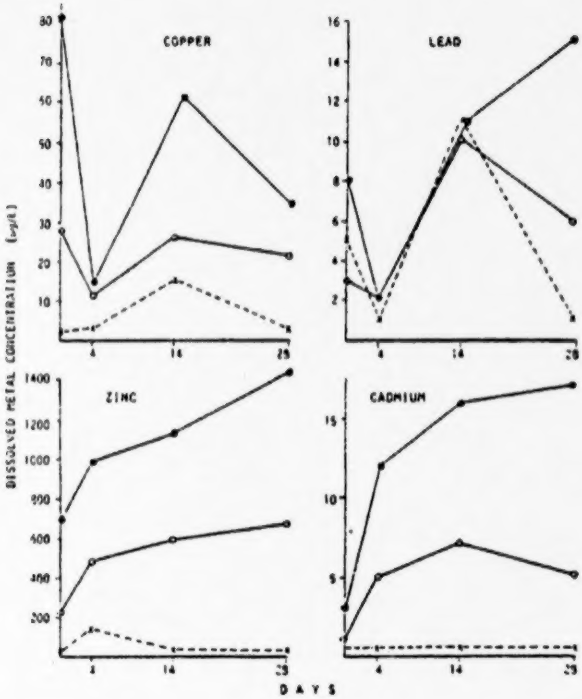

Figure 3. Metal concentration in freshwater from bioaccumulation experiments.

●——● 100%

○——○ 50/50 Sediment Control

x------x Control

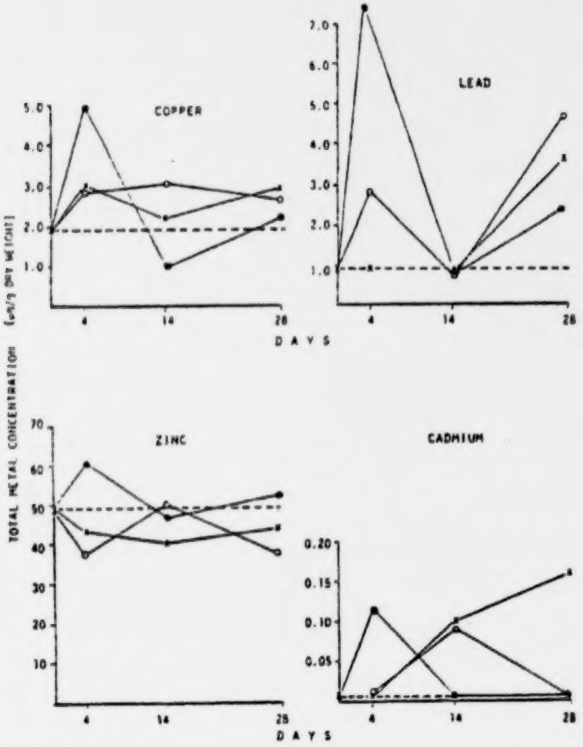

Figure 4. Metal concentration in sculpin (*Cottus asper*) muscle tissue from bioaccumulation experiments.

●——● 100% Tailings
o——o 50/50 Sediment Tailings
x——x Control
-------- Background

TABLE 5

METAL CONCENTRATIONS IN BIOTA COLLECTED FROM BUTTLE AND SURROUNDING LAKES

SPECIES	LOCATION/LAKE	AVERAGE FISH LENGTH* (mm)	WET TISSUE WEIGHT (g)	% MOISTURE	METALS (µg/g dry weight)			
					Cu	Pb	Zn	Cd
Sculpin (*Cottus asper*)	2 - Buttle	109	5.76	77.4	1.54	1.28	56.9	0.09
	4 - Upper Campbell	105	4.27	78.6	2.54	1.58	56.3	0.06
	6 - Campbell	90	4.08	77.1	2.32	2.18	89.3	0.07
	7 - John Hart	84	4.01	76.6	2.49	1.39	60.8	0.07
	8 - Upper Quinsam	79	4.13	76.1	1.73	1.01	38.1	<0.05
Plankton	1 - Buttle	-	-	89.9	174	52.5	570	3.27
	3 - Upper Campbell	-	-	89.2	352	657	1290	53.3
	5 - Campbell	-	-	89.5	81.5	75.2	774	49.4

*Fish length and analysis based on composite of 3 fish

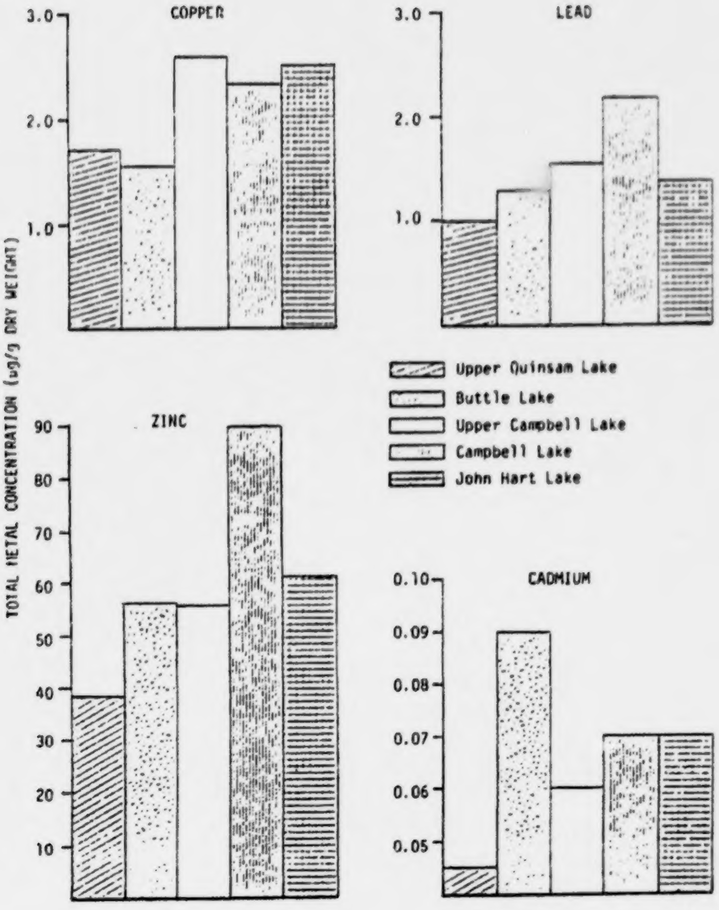

Figure 5. Metal concentration in sculpin from Buttle and surrounding lakes.

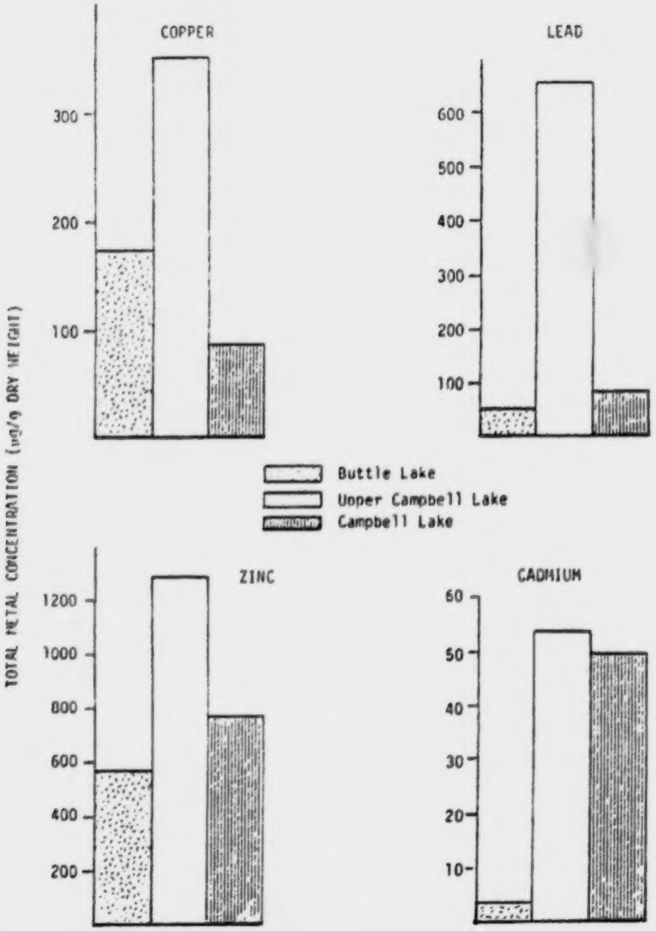

Figure 6. Metal concentrations in plankton from But[illegible]e and surrounding lakes.

tailings, highest zinc levels were obtained in 100% tailings (54.9 µg/g), while lead (4.75 µg/g) and copper (2.65 µg/g) were highest in the 50:50 mixture after 28 days. In general, values obtained in the present study are within the range for tissue levels reported in the literature. Brungs et al. (1975) found copper levels in gill, kidney and liver tissue of Brown bullheads (*Ictalurus nebulosus*) ranged from 10 to 116 µg/g dry weight after 30-day exposures to copper concentrations ranging from 27 - 104 µg/L. Rainbow trout (*Salmo gairdneri*) exposed to 18 µg/L lead in water for 32 weeks reached carcass levels of 1.0 µg/g wet weight (Hodson et al. 1978). Delisle et al. (1975) found peak whole body Cu, Pb, Zn, and Cd levels in catfish to be 125.0, 50.0, 4.0 and 0.8 µg/g dry weight, respectively, when exposed to contaminated sediment for 5 months. In this same study, tissue metal levels increased up to 2 months, declined then leveled off. Weiner and Giesy (1979) found muscle tissue levels of Cd, Cu, and Zn in bluegill exposed to a highly organic, soft water pond to be 0.0092, 1.28 and 34.8 µg/g dry weight, respectively.

Bioavailability, toxicity and accumulation of metals in aquatic biota are governed by factors such as the chemical nature of the environment, form of the dissolved metal and metabolism and excretion processes of the organism (Part and Svanberg 1981; Weiner and Giesy 1979; Jenne and Luoma 1977). In the present study, copper, lead and zinc bioaccumulated to a limited extent in muscle tissue after exposure to tailings or a tailing sediment mixture. It would appear that if significant uptake occurs, it is over a much longer time frame than the 28 days our study permitted. Also, uptake may be reflected more quickly in other tissues besides muscle tissue such as gill, liver, or kidney as demonstrated by Hodson et al. (1978).

Elevated zinc, lead and cadmium levels in water overlying the tailings suggest that these metals are available for uptake in freshwater species. The peak in tissue levels after 4 days exposure followed by a decline suggested rapid, initial uptake by the sculpins with a subsequent regulating of metal body burdens. Long-term uptake studies (i.e., 3 - 6 months) with examination of accumulation in individual organs and various biochemical indicators of metal stress would further define location and degree of uptake by target species.

Bioaccumulation in Resident Biota

Metal levels in sculpins and plankton from Buttle and surrounding lakes are listed on Table 5 and illustrated in Figures 5 and 6. Generally, levels of all metals were higher in sculpins collected from lakes downstream of the tailings outfall (Buttle, Upper Campbell, and John Hart Lakes) compared to Upper Quinsam Lake which served as a control. Northcote et al. (1975) found metal levels in prickly sculpins (*Cottus asper*) from Fraser River, B.C. of 21 ppm Zn and 0.74 ppm Cu (wet weight), considerably higher than those found at Buttle Lake. An important assumption in the comparison of metal concentrations in fish tissues is the similarity of exposure time and growth rates of the fish population sampled (McFarlane and Franzen 1980). While fish length in the present study was similar for each of the lakes (range 79 - 109 mm), age was not determined.

A particularly significant finding from the field sampling was that levels of all metals were considerably higher in plankton than sculpins from the same lake (Table 5). This result is consistent with the findings of Jackson et al. (1980) who concluded that plankton were one of the most important ecological components in accumulation of soluble metals from the water column. Future studies in the Buttle Lake system should focus on the role of plankton in the processes of metal bioaccumulation and biomagnification.

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PREDICTION OF ORGANIC CONTAMINANT AQUATIC TOXICITY UTILIZING INTRAPERITONEAL INJECTIONS AND STRUCTURE-ACTIVITY RELATIONSHIPS

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SMITH, I.R., and G.R. CRAIG. 1981. Prediction of organic contaminant aquatic toxicity utilizing intraperitoneal injections and structure-activity relationships. Can. Tech. Rep. Fish. Aquat. Sci.

Fifteen compounds (chlorinated and substituted benzenes, chlorinated phenols, ethylenes and other hydrocarbons) were injected into the peritoneal cavity of rainbow trout (*Salmo gairdneri*) and LD50s determined. Mean LC50s of the test compounds reported in the literature were correlated with octanol-water partition coefficients (log P) and IP LD50s to develop the following relationships:

1) LC50 mM/L = LD50 mM/kg x 0.005
2) Log LC50 mM/L = 1.08 - 0.88 log P
3) Log LC50 mM/L = 0.16 - 0.68 log P + 0.57 log LD50 mM/kg

The final equation modelled LC50s to within one order of magnitude which meets the basic requirements of a screening test. Combining the biological and chemical attributes of acute LD50 and theoretical log P values provides a simple, inexpensive and rapid preliminary evaluation of environmental organic contaminants in the hazard assessment process.

Key words: Aquatic, toxicity, organic, prediction, intraperitoneal, structure-activity.

SMITH, I.R., AND G.R. CRAIG. 1981. Prediciton of organic contaminant aquatic toxicity utilizing intraperitoneal injections and structure-activity relationships. Can. Tech. Rep. Fish. Aquat. Sci.

Quinze composés (benzines chlorés et substitués, phénols chlorés, éthylènes et autres hydrates de carbone) ont été injectés dans la cavité péritonéale de truites arc-en-ciel (*Salmo gairdneri*) et les LD50 ont été déterminés. Les LC50 moyens publiés pour ces composés ont été comparés avec les coefficients de partition octanol: eau (log P) et les IP LD50 pour développer les équations suivantes:

1) LC50 mM/L = LD50 mM/kg x 0.005
2) Log LC50 mM/L = 1.08 - 0.88 log P
3) Log LC50 mM/L = 0.16 - 0.68 log P + 0.57 log LD 50 mM/kg

L'équation finale s'approche à un ordre de grandeur de LC50, ce qui satisfait les exigences de base d'un test de triage. En combinant les attributs chimiques et biologiques de la valeur précise de LD50 et les valeurs théoriques de log P, on obtient une évaluation préliminaire simple, rapide et bon marché des contaminants organiques de l'environnement dans le procédé de détermination hasardeux.

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INTRODUCTION

Traditional approaches to determining the aquatic toxicity of organic contaminants are extremely expensive and time consuming. This is, in part, due to the inherent insolubility of many organics and the necessity of measuring exposure concentrations. Structure-activity relationships have been developed to predict bioaccumulation of organic compounds (Veith et al. 1979) and acute mortality in fish (Zitko 1975; Schultz et al. 1978; Konemann 1980). While acute toxicity is a rare occurrence in receiving waters, it is the first step in the process of developing a chronic and sublethal profile on organisms and facilitates the use of application factors for estimating more sensitive responses. Structure-toxicity relationships can simplify the examination of the thousands of organic contaminants and their potentially harmful effects on aquatic biota. The addition of integrative biological data would improve the reliability of developed models.

Direct injection of chemicals or toxicants is one of the simplest and most reliable means of dosing organisms. The injection of fish with toxicants to measure responses is becoming more common as in such studies with methyl mercury (Kendall 1975, 1977), lead (Ozoh 1979) and napthalene (Varanasis et al. 1979). The adoption of this technique with selection of class related compounds, as in conventional structure-toxicity studies, has been followed in this project. It is assumed that compounds are absorbed from the peritoneal cavity via the bloodstream and redistributed to various target organs for detoxification, excretion or storage.

Intraperitoneal (IP) injection of rainbow trout with organics was investigated as an acute toxicity screening procedure to provide information on pure compounds. The generated LD50 and literature LC50 values of the test compounds were correlated with log octanol-water partition coefficients (log P) to develop predictive relationships useful in hazard assessment protocols.

METHODS AND MATERIALS

Certified specific pathogen free rainbow trout (*Salmo gairdneri*) averaging 35g were held in 8°C well water (Wildcat Trout Farm, Thamesford, Ontario), and upon transfer to the laboratory were acclimated to 15°C at 1°C /day. Prior to testing, fish were held at 15°C for a minimum of one week in dechlorinated and ultraviolet sterilized Toronto tap water (Table 1) and were fed Ewos #4 trout food at 2% wet weight daily. Holding (700 L) and observation (95 L) tanks received 30 and 15 replacement volumes daily, were aerated to maintain dissolved oxygen > 8 mg/L, received fluorescent and natural lighting and were surrounded by black plastic screen to reduce visual disturbances.

Fish were randomly selected in groups of 10, anaesthetized in 50 mg/L phenoxy-ethanol or 100 mg/L ethyl-m-amino benzoate methanesulphonate (MS 222) and weighed. The ventral surface of the fish was dried, the chemical carrier solution (1 mL/100 g wet weight) injected into the peritoneal cavity in the area of the pyloric caeca. The body was pierced anterior to the pelvic fins and the body wall lifted with the point of the needle (27 gauge) prior to injection in order not to pierce the stomach.

A small plug of silicon stopcock grease (Dow Corning) was applied to seal the injection hole and the fish were transferred to the observation tanks after recovery from the anesthetic.

Chemicals (analytical grade) were dissolved in cod liver oil (Drug Trading Company of Canada Ltd., Lot #151281) within a carcinogen type glove box (Labconco Co.,) vented through activated charcoal, and stored in rubber capped injection bottles (Wheaton). Analysis of cod liver oil indicated 1.6 mg/L PCB's, 350 μg/L DDT, 420 μg/L DDD, 280 μg/L op'-hexachloride, 50 μg/L hexachlorobenzene, 150 μg/L chlordane, 35 μg/L hepatochlorepoxide and 25 μg/L dieldrin as contaminant residues. No analysis for test compound levels were performed.

A logarithmic series of doses for each compound were tested with a cod liver control. Mortality observations were repeated every 24 h for 96 h or until no further mortalities occurred. The LD50 values were calculated by probit analysis (Finney 1971), subjected to regression analysis and ANOVA after Ostle and Mensing (1975).

Aqueous exposure lethal values (LC50's) for the test chemicals were obtained from the literature and water-octanol partition coefficients were calculated according to Leo et al. (1971) and Leo (1975).

Table 1. Chemical profile of dilution water.

Parameter	Quantity (mean)	Standard Deviation
Free Ammonia (NH_3)	0.01 mg/L	0.005
Total Kjeldahl Nitrogen	0.13 mg/L	0.10
Total Nitrite (NO_2)	0.001 mg/L	0.001
Total Nitrate (NO_3)	0.45 mg/L	0.05
Hardness (as $CaCO_3$)	135.0 mg/L	10.0
Alkalinity (as $CaCO_3$)	85.0 mg/L	5.0
Total Residual Chlorine	36.0 g/L	20.0
Conductivity	340.0 mohs/cm	10.0
pH	7.6	0.2
SO_4	31.0 mg/L	N.A.*
Cl	29.0 mg/L	N.A.*
Na	13.0 mg/L	N.A.*
K	1.5 mg/L	N.A.*
Ca	38.0 mg/L	N.A.*
Mg	7.5 mg/L	N.A.*
Total Coliform (per 100 mL)	4.0	10.0
Aeromonas hydrophia (per 100 mL)	4.0	10.0

* Not available, due to insufficient number of samples.

RESULTS

Octanol-water partition coefficients for the test chemicals were either calculated (Table 2) or obtained from the literature. Acute lethal concentrations (LC50's) were also gathered from the literature for fathead minnows and bluegill sunfish as well as rainbow trout (Table 3). The limited data available for trout required that a mean LC50 value be calculated, regardless of species, to obtain a "representative" fish lethality value for each compound.

The LD50 values (mM/kg) for the 15 test compounds (Table 4) ranged over three orders of magnitude. The majority of the mortalities (90%) occurred within 20 h and 97% occurred within 72 h. No control mortalities occurred during the course of the 21 tests and several control fish injected with three times the normal volume of cod liver oil survived for 10 days without feeding, suffering no observable effects.

Cholorinated phenols were the most toxic group (LD50 0.09-0.6 mM/kg) with the remaining compounds producing LD50's in the range of 10-50 mM/kg. Tetra- and pentachlorobenzene were the least toxic proving to be insoluble in the carrier above 7.5 and 5 mM/kg thus the response was recorded as loss of equilibrium and buoyancy (ED50). The highest dose of tetra- (15 mM/kg) and pentachlorobenzene (10mM/kg) was achieved by doubling the injection volume which produced 10% mortality and 90-100% immobility in surviving fish exposed to both compounds. Fish recovered normal behaviour and appearance within 120 h.

Table 2. Partition coefficients (Log Octanol/Water) for the test compounds.

Compound	Experimental	Calculated
Benzene	2.13 (1)	2.13 (2)
Toluene	2.73 (1)	2.47 (3)
Xylene	2.77, 3.15, 2.77 (4)	3.27 (3)
1,2-dichlorobenzene	3.38 (5)	3.37 (2)
1,2,4-trichlorobenzene	4.2 (6), 4.7 (7)	4.26 (2)
1,2,4,5-tetrachlorobenzene		4.97 (2)
Pentachlorobenzene		5.68 (2)
Phenol	1.46 (1)	1.5 (2)
2,4,5-trichlorophenol	3.37 (8)	3.27 (2)
Pentachlorophenol	5.01 (8)	4.51 (2)
Carbon tetrachloride	2.64 (9)	2.84 (2)
Hexachlorobutadiene		4.10 (2)
1,1-dichloroethylene	0.73 (10)	1.74 (3)
Trichloroethylene	2.29 (10)	2.11 (3)
Tetrachloroethylene	2.53 (10)	2.48 (3)

1) Fujita et al. 1964
2) after Leo et al. 1971
3) after Leo, 1975
4) Anon, 1978
5) Tichy et al. 1971
6) Vieth et al. 1979
7) Ozburn et al. 1980
8) Church et al. 1971
9) Macy, 1948
10) Radding et al. 1977

Table 3. Test compound LC50 values (mM/L) for rainbow trout (RBT), fathead minnows (FHM), and bluegill sunfish (BGS).

Compound	RBT	FHM	BGS	Mean
Benzene		.41 (1)	.28 (1)	.342
		.43 (2)	.25 (3)	
Toluene		.31 (4)	.13 (4)	.298
		.41 (1)	.27 (1)	
		.37 (3)		
Xylene		.27 (1)	.20 (1)	.235
1,2-dichlorobenzene		.18 (5)	.038 (4)	.109
1,2,4-trichorobenzene		.018 (4)	.017 (4)	.0175
1,2,4,5-tetrachlorobenzene		.0072 (4)	.0072 (4)	.0072
Pentachlorobenzene	.0098 (4)	.00099 (4)	.00099 (4)	.00099
Phenol	.098 (6)	.71 (4)	.25 (1)	.266
	.123 (7)	.302 (12)	.15 (14)	
	.094 (8)	.352 (1)	.63 (6)	
	.053 (9)	.318 (13)		
	.082 (10)	.491 (8)		
	.079 (11)			
2,4,5-trichlorophenol	.0045 (15)		.0023	.00343
Pentachlorophenol	.00075(15)	.00127 (4)	.00072(18)	.00121
	.00029(16)	.00083(12)	.00014(20)	
	.00097(16)	.0058 (13)		
		.0008 (17)		
		.00079 (3)		
Carbon tetrachloride		.81 (5)	.17 (4)	.49
Hexachlorobutadiene				.0034
1,1-dichloroethylene		1.4 (22)	.76 (4)	1.08
Trichloroethylene		.41 (23)	.34 (4)	.375
Tetrachloroethylene		.12 (23)	.077 (4)	.098

1. Pickering & Henderson 1966
2. Shell Chemie 1975
3. Turnbull et al. 1954
4. Anon, Water Quality Criteria 1978
5. Dawson et al. 1977
6. Cairns et al. 1978
7. Fogels & Sprague 1977
8. DeGrave et al. 1980
9. McLeay 1976
10. Brown et al. 1967
11. Mitrovic et al. 1968
12. Phipps et al. 1981
13. Ruesink & Smith 1975
14. Cairns & Schier 1959
15. Huttula et al. 1981
16. Davis & Hoos 1975
17. Adelman & Smith 1976
18. Pruitt 1977
19. Bentley 1975
20. Inglis & Davis 1972
21. Leewangh et al. 1975
22. Dille et al. 1979
23. Alexander et al. 1978

Table 4. Intraperitoneal LD50 and ED50 values with 95% confidence limits for the test compounds injected into rainbow trout.

Compound	LD50 or ED50 (mM/kg)	U.L.*	L.L.**	Slope
Benzene	44.1	54.7	35.5	6.05
Toluene	48.9	65.6	36.5	10.4
Xylene	38.2	48.9	29.8	11.2
1,2-dichlorobenzene	54.5	203.9	14.9	1.7
1,2,4-trichlorobenzene	14.8	18.1	12.1	9.9
1,2,3,4-tetrachlorobenzene	+10.6	10.9	10.4	4.6
Pentachlorobenzene	+ 3.4	6.3	0.1	4.5
Phenol	5.9	7.5	4.8	1.6
1,3,5-trichlorophenol	0.6	0.8	0.5	7.1
Pentachlorophenol	0.09	0.11	0.08	10.3
Carbon tetrachloride	40.3	59.1	27.5	2.85
Hexachlorobutadiene	8.3	59.3	1.2	1.87
1,1-dichloroethylene	26.5	31.5	22.3	7.84
Trichloroethylene	34.0	43.8	26.5	2.98
Tetrachloroethylene	37.5	47.0	29.9	3.83

* Upper 95% Confidence limit
** Lower 95% Confidence limit
+ ED50 (mM/kg)

DISCUSSION

A good screening test should be simple, reliable and economic. One of the most difficult and expensive aspects of hazard assessment is chemical analysis of exposure concentrations and determination of partition coefficients (log P). The advantage of calculating the log P for compounds, instead of determining values analytically, is clear for screening tests. In the same way the preparation of solutions of compounds for injection avoids the need for chemical analysis due to the accuracy of the dilution process. These two approaches have been combined to develop a simple, inexpensive method of screening insoluble compounds for aquatic toxicity.

Carrier Contamination

The use of fish oil carriers inherently introduces a variety of environmental contaminants. Cod liver oil was selected to provide biological compatibility. Preliminary testing of some compounds using contaminant free corn oil has produced similar results to the cod liver oil.

The effects of contaminants in the carrier would be expected to be maximal for the pentachlorophenol tests due to the larger relative volume of carrier required. The majority of the organic compounds were administered in concentrations up to four orders of magnitude greater than that of the contaminants in the cod liver oil, consequently the effects of carrier contamination would be expected to be minimal in this acute testing format.

The maximum dose of PCB's (the dominant contaminant) which a fish would receive due to injection in this study would be 16 μg/kg, well below levels (900 μg/kg) in some fish food or levels (14 mg/kg/day) inducing hepatic mixed function oxidases (Addison et al. 1979). Despite this apparent lack of effect, especially when dealing with acute mortality, it is recommended that uncontaminated carriers be used (particularly when evaluations lead to sublethal investigations).

Log P - Calculated vs Measured

The practical and economic advantages of using calculated instead of measured log P values (which are dependant on analytical variability introduced by the choice of method) is obvious but cannot be considered alone. Comparisons of the log P derivation (calculated vs measured) was made by correlating values with the LD50 data generated by this study. While correlations for the two relationships were similar, the coefficient was better for theoretical log P values and were consequently utilized in this report. Using calculated log P values for screening evaluations not only circumvents the need for chemical analysis and the dependence on literature values but also assures the reproducibility of the approach among laboratories.

Estimating LC50 using log P and LD50

Regressions between LC50 and log P for different classes of compounds appears to be dependent on the chemical characteristics of respective classes (Fig. 1). However, treating all data without distinction as to class produces as strong or a stronger correlation coefficient than individual treatments. The relationship developed from data averaging responses from three species,

$$\text{Log LC50 mM/L} = 1.08 - 0.88 \log P \quad (r = 0.81;\ p < 0.05)$$

agrees well with the LC50 estimate generated by Konemann (1980) with chlorotoluenes, benzenes, hydrocarbons, alcohols and glycero-derivities (Log LC50 mM/L = 1.8 - 0.87 log P, $r = 0.81$; $p = 0.05$). Data generated by both equations are within an order of magnitude of the other (factor of six) with the latter study using guppies tested under static renewal conditions. Other factors (Zitko 1975; Zitko et al. 1976; McLeese et al. 1979; Schultz et al. 1980) have also demonstrated the linearity of relationships developed between the LC50's of dissimilar classes of compounds and respective log P's.

The relationships developed between LD50's and log P (Fig. 2) differ somewhat in that slopes are less pronounced for each chemical class and the correlation coefficients are weaker. However, the relative toxicities of the classes are similar and the regression developed from all the data is stronger. One of the difficulties inherent with both approaches to data interpretation is that when dealing with classes of compounds the amount of data is limited by the number of available chemical representatives for each class. Combining classes improves relationships by increasing data points.

Since both LC50 and LD50 can be related to log P it is not unreasonable to establish a relationship between the two (Fig. 3), which simplifies to the following linear function:

$$\text{LC50 mM/L} = \text{LD50 mM/kg} \times 0.005$$

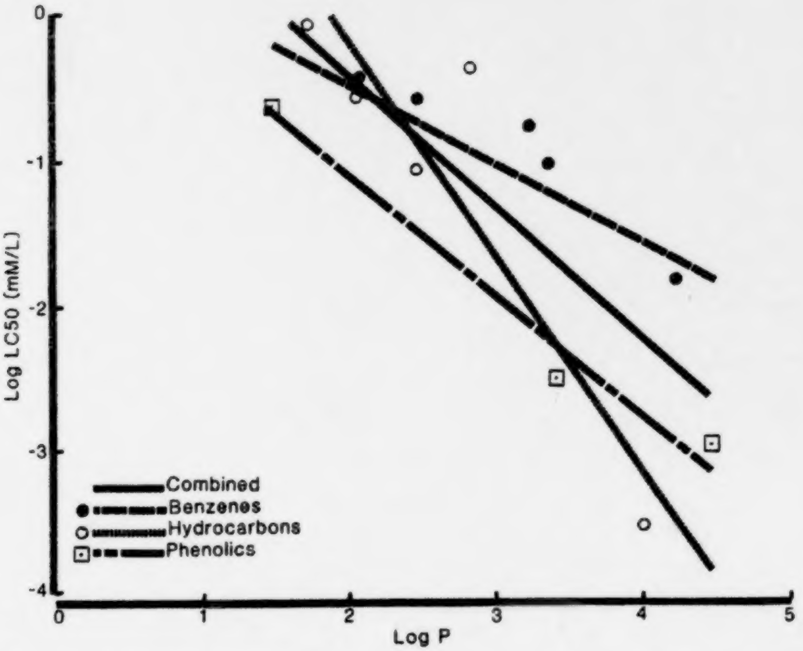

Figure 1: The relationships for the tested compounds aqueous toxicity and partition coefficients.

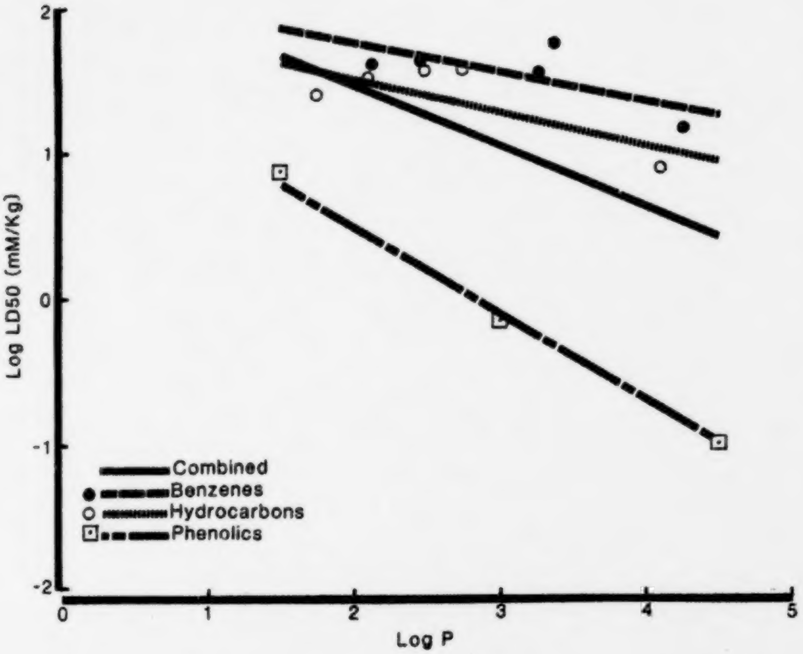

Figure 2: The relationships for the tested compounds injection toxicity and partition coefficients.

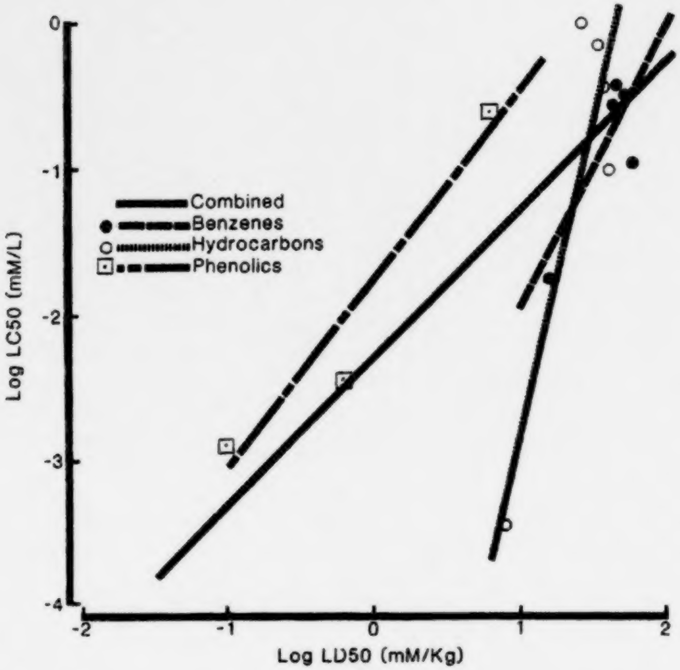

Figure 3: The relationships for the tested compounds injection and aqueous toxicities.

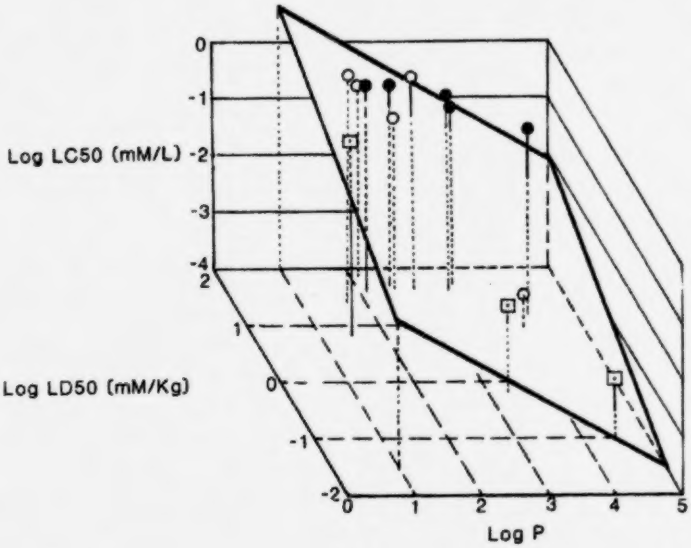

Figure 4: A multiple linear relationship for the tested compounds aqueous and injection toxicities and their partition coefficients.

This relationship appears to contradict the understanding of "effective dose" and "exposure concentration" since one normally expects the LD50 to be less than LC50. The method of toxicant administration and selection of carrier has long been known to affect the degree of organism response. However, the amount of compound entering via the gill is sustained for the exposure period and must continually affect the site(s) of action compared to the injection exposure which assaults the organism during the initial test period and then diffuses into the blood stream and/or sequesters in adjacent lipid areas. The dynamics and distribution of these compounds in fish is not well documented and will undoubtedly explain response differences. Greater consideration, is being given to expressing toxicant dose, regardless of administration, according the area subtended by the concentration/time curve in target tissues or in the circulatory system. In this case, the toxicant dose due to sustained exposure in water may well be greater (by two orders of magnitude) than that experienced by a single injection of a lipophilic compound that could migrate to and be isolated in local fat reserves.

This study illustrates that some compounds can vary considerably from the regression lines developed from experimental data. The addition of a second, simple, reproducible coordinate such as log P would improve the reliability of the LC50 estimate (Fig. 4). A regression relationship to estimate LC50 based on LD50 data and log P can be expressed as:

$$\text{Log LC50 mM/L} = 0.16 - 0.68 \log P + 0.57 \log (\text{LD50 mM/kg})$$

Both Log P and LD50 can be developed without analytical support and the LC50 can be estimated within an order of magnitude for screening purposes.

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BIOACCUMULATION OF MERCURY BY ATTACHED ALGAE IN ACID STRESSED LAKES

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The capacity of algae to accumulate metals from water along with the observation of locally abundant growth of attached algae in acid-stressed lakes led to the suggestion that this algal community could be used to monitor mercury in acidic lakes. Unlike copper, zinc and nickel, mercury has been shown to bioaccumulate most readily as the methyl mercury species. Furthermore, there is considerable interest in and concern over elevated mercury levels in fish from acidic lakes.

Two recent studies have each shown that for a number of lakes in the same geographical area the lake water pH showed a significant correlation with the mercury concentration in fish muscle tissue. It has been suggested that the increase in mercury concentration in fish at lower pH may be related to a number of chemical and biological factors including changes in chemical speciation of mercury and decreased growth rates of fish, but the relative importance of these factors remains unknown.

The actual mercury content of substrate-grown algae showed remarkably low within-site variation, while the range of mercury in algae from different lakes was rather large - 40-145 ppb.

The selected lakes are in the same general region of the Canadian Precambrian Shield, far from any point source of mercury. Yet there is a significant inter-site variation in mercury content, and a very good relationship between total mercury concentration in the algae and in the yearling perch reported by Suns et al. (1980) from the same lakes (r = 0.93).

These properties in substrate-grown algae show their potential as excellent and as yet little used monitors of mercury dissolved in water.

Key words: Mercury; methyl mercury; algae; acid lakes; biological monitor; mercury bioaccumulation.

STOKES, P.M., S.I. DREIER, M.V. FARKAS, and R.A.N. McLEAN. 1981. Bioaccumulation of mercury by attached algae in acid stressed lakes. Can. Tech. Rep. Fish. Aquat. Sci.

L'habilité qu'ont les algues d'accumuler les métaux présents dans l'eau ainsi que l'observation d'algues fixes abondantes localement dans les lacs acides nous a suggéré l'idée que cette communité d'algues pourrait être utilisée pour mesurer la quantité de mercure présente dans les lacs acides. Contrairement aux cuivre, zinc et nickel, il a été démontré que le mercure s'accumule facilement dans les tissus vivants sous différentes formes de méthyl de mercure. De plus, il y a beaucoup d'intérêt et d'inquiétudes au sujet des niveaux élevés de mercure trouvés dans les poissons de lacs acides.

Deux études récentes ont chacune démontré que, pour plusieurs lacs d'une même région géographique, le pH de l'eau est directement relié à la concentration de mercure dans les tissus musculaires des poissons. Il a été suggéré que, à un pH plus bas, l'augmentation de la concentration de mercure dans les poissons soit reliée à différents facteurs chimiques et biologiques comprenant les changements dans la spéciation du mercure et le taux de croissance plus lent des poissons, mais leurs importances relatives sont toujours inconnues.

La quantité de mercure mesurée dans les algues cultivées sur substrat montre une variation remarquablement petite à l'intérieur du même site alors que la variation dans la quantité de mercure des algues de différents lacs est plus grande - 40-145 ppb.

Les lacs choisis sont dans la même région générale du bouclier canadien précambrien, éloignée de toute source de mercure. Cependant, la variation de la quantité de mercure entre les sites est significative et il y a une très bonne correspondance entre la concentration totale de mercure dans les algues et les jeunes perches agées d'un an et provenant de ces mêmes lacs, tel que Suns et al. (1980) le reportent (r = 0.93).

INTRODUCTION

The capacity of aquatic plants to accumulate metals from water and/or sediments has been demonstrated many times for field collections, usually from metal-contaminated sites (e.g. Fuller and Averett 1975; Hutchinson and Czyrska, 1974) and for laboratory-grown cultures (e.g. Steeman Nielsen et al. 1969; Sakaguchi et al. 1977; Burkett 1975). In spite of the almost complete absence of species in common, both field and laboratory studies indicate that some generalisations can be made concerning the process of metal uptake by planktonic and attached algae. Uptake is normally a concentration-dependent, passive process, involving both cell wall binding and intracellular uptake involving membrane transport. For metals such as copper, zinc, nickel and cadmium, the form available for uptake is normally the "free" or hydrated ion.

The possibility therefore exists that algae can be used as monitors for metals. This idea has been discussed in the literature and potential advantages include the following:

a) The high concentration factors attained by plants mean that the plants effectively improve the detection limit. (Dietz 1972)
b) The plant is selective, i.e. takes up the biologically available form(s) of a metal and therefore gives a better indication of potentially damaging levels than direct chemical analysis can give.
c) Like other biological monitors, plants can integrate the metal burden of a system over time. (Say et al. 1977)
d) Long term storage of dried plant material is feasible and samples can be retained for long periods, providing an historical catalogue as well as material for newly developed analytical techniques.

In the field, planktonic algae rarely produce sufficient biomass to be of practical use, but attached algae are more promising as they possess a number of attributes including rapid growth, high biomass production and sessile habit. Their capacity to grow on inert artificial substrates means that they can provide uniform material of known age.

The recognition of these properties, along with the observation of locally abundant growth of attached algae in acid stressed lakes led to the suggestion that this algal community could be used to monitor mercury in acidic lakes. Unlike copper, zinc and nickel, mercury has been shown to bioaccumulate most readily as the methyl mercury species. There is considerable interest in and concern over elevated mercury levels in fish from acidic lakes (NRCC 1981). This situation appears to be distinct from one of actual mercury pollution, for the water and sediments of the lakes do not contain elevated levels of mercury.

For some time it has been suggested that there was an inverse relationship between the mercury concentration in fish muscle tissue and the pH of the water (Landner and Larsson 1972; Jernelov et al. 1975; Brouzes et al. 1977; Lithner 1978). However this proposed relationship and the possible mechanisms used to explain it (Brouzes et al. 1977; Jernelov 1980) were based on scanty data collected for other reasons. Two more recent studies (Suns et al. 1980 and Hakanson 1980) have each shown that for a number of lakes in the same geographical area the lake water pH showed a significant correlation with the mercury concentration in fish muscle tissue for yearling perch (Suns et al. 1980) and "1 kg" pike (Hakanson 1980). It has been suggested that the increase in mercury concentration in fish at lower pH may be caused by any or all of the following factors (McLean et al. 1979 and Tomlinson et al. 1980):

a) Chemical - (i) Increased scavenging of methyl mercury and other mercury compounds from the atmosphere by low pH aerosols, clouds and rain.
  (ii) Relatively higher production of the bioaccumulable mono-methyl mercury species at low pH compared with the more volatile dimethyl mercury.
  (iii) Increased desorption of mercury species from solids into the water and resulting increased bioavailability.
  (iv) Greater absorption of mercury from the atmosphere by low pH receiving water.
  (v) Decreased re-emission to the atmosphere from low pH water.

b) Biological - (i) Reproductive failure and decreased food supply for fish in acidified water.
  (ii) Increase in the amount of water passing over the gills of foraging fish with decreased food availability can result in greater methyl mercury uptake per unit weight increase.
  (iii) Accelerated biomagnification if piscivores are forced to feed on larger fish which have higher mercury content than small younger fish.
  (iv) Decreased biomass of fish per unit volume of water produces greater mercury per unit weight of remaining biomass.

There is very limited information on the actual mechanism by which fish and other aquatic biota accumulate mercury, and most studies deal with laboratory experiments or with field situations where mercury is at rather high concentrations. Since the phenomenon is so complex, and the observations relatively recent, it is not surprising that no studies have been reported which directly address or test the role of the various factors outlined in a(i)-(v) and b(i)-(iv). Furthermore, there are several major variables as well as pH which can affect the physiochemical and the biological conversions of mercury in aquatic systems (Fagerstrom and Jernelov 1972) and the uptake of mercury by biota (Hakanson 1980).

In the summer of 1981, in the course of a survey of lakes in South Central Ontario, an opportunity arose to carry out some preliminary experiments on the mercury content of algae in lakes which were variously showing low alkalinities and pHs generally between 5.0 and 6.0, and from which mercury in fish had already been measured by Suns et al. (1980).

The study was designed to determine the potential of filamentous algae as monitors of mercury in water, and to evaluate any relationships between several key parameters in water chemistry including pH, and mercury content of tissue. As well, it was anticipated that a system could be designed which might provide some insight into the mechanism of mercury accumulation in algae specifically but also in aquatic biota in general. Lakes were selected to provide a range of pH within the same geographic region. The immediate objectives were:

1) to sample filamentous algae from artificial as well as natural substrates from lakes having a pH between 5 and 6.
2) to determine if any relationship existed between the mercury content of algae and pH, or mercury content of the water.
3) to compare the substrate-grown with the natural algae in terms of total Hg content.
4) to determine if there were any relationship between mercury in fish and mercury in algae.
5) to determine the ratio of methyl to total mercury in algae from different sites.

METHODS

Site Selection

Lakes were chosen to include five which were common to the set described by Suns et al. (1980) as well as five others which were under study. Table 1 shows some of the chemical properties of the five lakes in which substrates were placed.

Table 1. Water chemistry for artificial substrate sites

Lake	pH	Alk.[1] µeq L^{-1}	D.O.C. mg L^{-1}	Cond.[2] 25°C µmhos cm^{-1}	Total reactive Ca mg L^{-1}	Total Fe mg L^{-1}	Total P (reactive phosphate) mg L^{-1}	Total N (reactive nitrate) mg L^{-1}
CY[3]	7.2	122.0	3.0	56	5.4	0.12	0.084 (0.066)	0.36 (0.005)
CB[4]	5.8-6.2	15.1	4.2	30	2.4	0.08	0.080 (0.056)	0.35 (0.005)
HY[5]	5.6-5.8	13.8	2.1	27	2.0	0.11	0.078 (0.063)	0.25 (0.005)
CN[6]	5.5	24.4	9.5	25	2.0	0.91	0.022 (0.013)	0.31 (0.025)
LD[7]	5.4	13.0	2.2	35	2.2	0.26	0.011 (0.001)	0.26 (0.005)

[1] Alkalinity
[2] Conductivity
[3] Cranberry Lake
[4] Chub Lake
[5] Heney Lake
[6] Crosson Lake
[7] Leonard Lake

Artificial Substrates

Artificial substrates were set in five lakes for a 25-day-period from July 2, 1981 to July 27, 1981. Eight plexiglass plates covered on one side with 'Mylar' drafting film, were set vertically in a slotted holder. The total area of each sheet of Mylar was 270 cm^2. Mylar had previously been found to be a suitable surface for growth and harvesting of attached algae (Dreier et al. 1980). The holder was placed in 0.5 - 1 m water in the lake, usually close to an inflowing stream where the natural growth of periphyton was most abundant. After 25 days, the material on each piece of Mylar was scraped onto a preweighed Uni-Pore polycarbonate membrane filter (0.4 µm pore, 47 mm diameter) using an acid-washed plexiglass scraper. Following filtration, filter and algae were placed in glass vials and dried to a constant weight at a maximum temperature of $70^{\circ}C$. All apparatus and containers used were glass or plastic and had been acid-washed and rinsed seven times with distilled water. Small subsamples were retained undried for algal identification.

In addition to the eight replicate samples harvested from the artificial substrate in each lake, two samples of periphyton growing on the natural substrate were harvested onto filters in the same manner. At the time of harvesting, at each artificial substrate site, pH was measured in the field, using an I.L. Portomatic meter. Inflection point alkalinity (MOE 1979) was measured within 12 h of collection. Water samples were collected for chemical analysis: one for nutrients, and two unfiltered (replicates) for Hg. These were preserved immediately with nitric acid and potassium dichromate as described in Brouzes et al. (1977). Sediments were collected for the same sites, using an Eckman dredge and were sealed in plastic bags and frozen until analysed.

Algae Attached to Natural Substrates

In September 1981, samples of attached algae were harvested directly from natural substrates. The mat was removed with plastic forceps and material other than algae (bark, twigs, etc.) was excluded as much as possible. Duplicate samples were collected, stored in acid-washed jars and dried as described for the collections in July. The pH of the water was measured in the field using a Fisher digital meter, and in the laboratory on a Radiometer model PAM-64 meter.

Mercury Analysis of Water, Sediment and Algae

Dried sediments were weighed accurately and digested based on the method of Floyd and Summers (1975) (McLean and Farkas, unpublished). Algae were similarly prepared and digested based on the method of Bishop et al. (1975).

For total mercury, the waters and digests were analysed by flameless atomic absorption spectrophotometry. Methyl mercury was determined using gas liquid chromatography, based on Cappon and Smith (1980).

RESULTS

Concentrations of mercury in water were close to the detection limit (0.01 $\mu g\ L^{-1}$ - 0.03 $\mu g\ L^{-1}$) and sediment concentrations were also quite low, from 30 - 150 ng g^{-1} (Table 2).

The growth on the artificial substrates was dominated by the green filamentous algae *Mougeotia* spp. and *Spirogyra* spp. with some differences in species composition between sites. The total mercury concentrations for the algae are shown in Table 3. The values showed very low within-site variation, while the range of mercury was quite large, from 40 - 145 ppb. The mercury concentrations for algae collected from natural substrates are shown in Table 4. These are in general in the same range and show quite high concentration factors but show more within-site variation. Furthermore there is poor agreement between the July and August collections. There were insufficient natural algae for mercury analysis in Cranberry and Leonard Lakes. Clearly there was no correlation between pH of the water or of the sediment and mercury content for either the substrate-grown or the naturally growing algae.

The five lakes for which substrate-grown algae were analysed are part of the sample set for which Suns et al. (1980) reported mercury in yearling perch. Their values are included in Table 3. These workers found significant correlations between mercury content and epilimnetic pH, aluminum and the lake drainage area/lake volume ratios, respectively.

When the mercury in fish was compared with the mercury in substrate-grown algae for these five lakes, there was a significant correlation, shown in Figure 1 ($r = 0.93$, $p = 0.02$). No comparable statistical comparison could be made for the algae on natural substrates because the small amounts of material limited the numbers of analyses. (Replicate samples were pooled for this material).

Table 2. pH and mercury content of water, and mercury in sediments

Lake	pH	Water Hg $\mu g\ L^{-1}$	Sediment Hg ng g^{-1}
Cranberry	7.2	<0.01	30
Chub	5.8-6.2	<0.01	30
Heney	5.6-5.8	<0.01	30
Crosson	5.5	0.03	125
Leonard	5.4	0.01	150

Table 3. Total mercury in algae and in fish from five lakes in south central Ontario

Lake	pH	Alk. µeq L^{-1}	Hg µg g^{-1} dry weight in substrate grown algae	Hg µg g^{-1} wet weight* in yearling perch
Cranberry	7.2	122.0	0.04 (0.018)	0.059 (0.004)
Chub	5.8-6.2	15.1	0.138 (0.013)	0.197 (0.038)
Heney	5.6-5.8	13.8	0.145 (only 2 samples)	0.171 (0.022)
Crosson	5.5	24.4	0.125 (0.01)	0.175 (0.02)
Leonard	5.4	13.0	0.09 (0.005)	0.083 (0.014)

Means of 4 samples ( ) standard deviation.

*From Suns et al. (1980).

Table 4. Mercury in algae from natural substrates

	Total Hg µg g^{-1} Dry Weight (pH)	
Lake	July Collection	August Collection
Red Chalk	-	0.27 (6.5)
Chub	0.09 (5.9)	0.33 (5.8)
Fawn	-	0.09 (5.8)
Heney	0.08 (5.7)	0.09 (5.8)
Crosson	0.18 (5.5)	0.05 (5.3)
Harp	-	0.19 (5.4)
Swan	-	0.12 (4.6)
Dickie	-	0.12 (4.5)

- no data

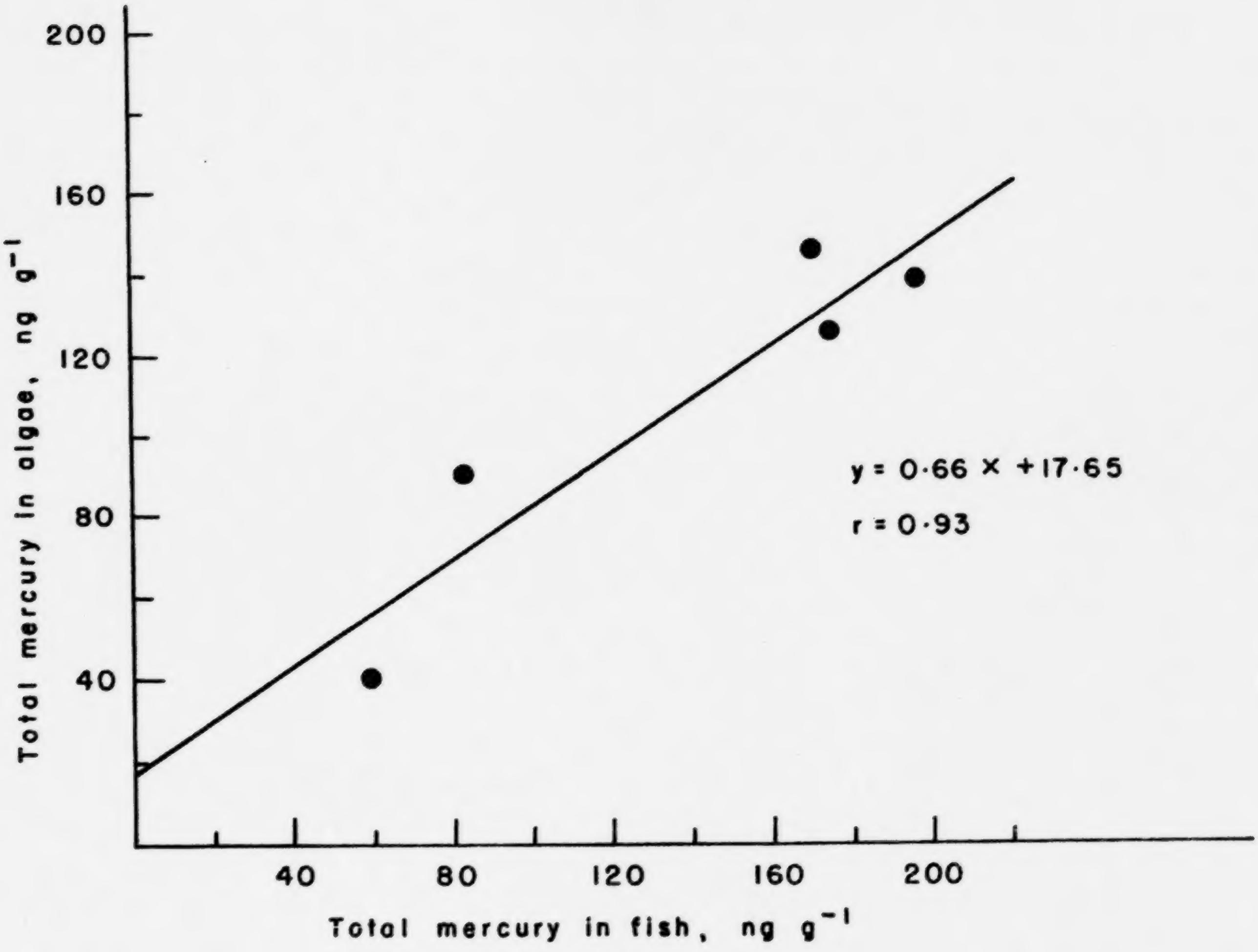

Figure 1

Relationship between mercury in yearling yellow perch (Suns et al., 1980) and substrate grown algae from five lakes

DISCUSSION

The low intra-site variation and the large range in the mercury concentration in substrate-grown algae show their potential as excellent and as yet little used monitors of mercury in lakes. The selected lakes are in the same general region of the Canadian Precambrian Shield far from any point source of mercury. The correlation between mercury in algae and fish was unexpectedly good, and it is tempting to suggest that some common mechanism was in operation. There is evidence which indicates that for higher mercury concentrations at least, monomethyl mercury is the form which is taken up by fish although this has not been tested for fish in acidic lakes of low concentration. The mercury in the substrate-grown algae in Cranberry and in Chub lakes (i.e., one alkaline and one slightly acidic lake) was almost 100% methyl mercury (Table 5).

Table 5. Methyl mercury concentrations in algae

Lake	pH	Type of Algae	Methyl Mercury ($\mu g\ g^{-1}$)	Total Mercury ($\mu g\ g^{-1}$)
Cranberry	7.2	Artificial Substrate	0.04	0.04
Chub	5.8-6.2	Artificial Substrate	0.12	0.14
Heney	5.6-5.8	Natural Substrate	0.05	0.09
Dickie	4.5	Natural Substrate	<0.05	0.12
Swan	4.6	Natural Substrate	<0.05	0.12

For the algae collected from natural substrates, the mercury content was very variable, and only a small proportion was in the methyl form (Table 5). This fact, plus the great variability and inconsistency of mercury content of natural algal communities would limit their use as monitors. The variability is not of course surprising since the algal collections were of unknown age and history, and inevitably included dead algae and empty cell walls as well as entrained material.

pH is only one of the variables which is likely to affect mercury uptake by aquatic biota. This is discussed at length by Hakanson (1980) who has devised a model which relates mercury in fish to function of pH of the water, mercury in sediment and the bioproduction index (nutrient status) of the sediment. Appropriate sediment parameters were not measured for the present study, nor for the Suns et al. (1980) study. However, Hakanson's model could be tested in the future for algae. It would be of interest to determine for example if Leonard Lake, which had the lowest pH of the set of five, but which had relatively low mercury levels in both algae and fish (Table 3) has some sediment characteristics which counteract or damp the expected effect of pH. Certainly the water chemistry of Leonard does not diverge from the expected ranges for any of the parameters measured (Table 1).

In the context of the present study, the pH dependence of mercury would first have to be established. A consideration of the effect of pH on the uptake of any metal by biota has to include the following aspects:

1) the chemical speciation and solubility of the metal in water is pH dependent;
2) the physiological condition of the organism is likely to be affected by pH of the water;
3) different forms of mercury are taken up at different rates, and;
4) with time, mercury may be lost from biota.

While 1) and 2) might be expected to favour mercury uptake at low pH (as shown for fish), 3) and 4) are complicating factors for natural communities of algae. Uptake of mercury includes true intracellular uptake, as evidenced by the loss of potassium associated with mercury treatment (Sheih and Barber 1973). Richardson et al. (1975) show a biphasic uptake for mercury into the alga *Pediastrum boryanum*, suggesting cellwall binding followed by intracellular uptake. Time-dependent losses of mercury from algal cells was shown by Ben-Basset and Mayer (1975) who suggested the release of elemental mercury. The mixed age and unknown physiological condition of algae collected from natural substrates therefore make interpretation of their mercury content quite complex. For the substrate-grown algae the situation is much more simple.

In conclusion, it is recommended that substrate-grown filamentous algae be investigated for their potential as rapid, inexpensive and convenient monitors of mercury, especially in lakes in the pH range 6.5 - 5.0 in which acidification is occurring and the algae are proliferating. Furthermore, while it is not essential to have a complete understanding of a mechanism before adopting a practical monitoring use for the algae, it is both feasible and desirable to investigate the chemical, physiological and biophysical aspects of mercury uptake into aquatic biota. The algae have already been isolated and cultured, and can provide convenient material for such studies in the laboratory and in the field.

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SHORT-TERM TOXICITY TESTS FOR ASSESSING ENVIRONMENTAL POLLUTANTS

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TREVORS, J.T., C.I. MAYFIELD, and W.E. INNISS. 1981. Short-term toxicity tests for assessing environmental pollutants. Can. Tech. Rep. Fish. Aquat. Sci.

Rapid methods for assessing toxicological effects of chemicals are needed to simplify the task of screening large numbers of potentially toxic chemicals. The first part of this paper outlines a method for studying the effect of sequence of exposure to toxicants in a bioassay using the bacterium *Pseudomonas fluorescens* as the test organism. Since toxicants often do not exert their full potential in single applications, a bioassay employing two sequential toxicant exposures, separated by a period of essentially no exposure, was developed. The response of the test organism to different toxicants was clearly dependent upon both the sequence of toxicant addition and the toxicant concentrations used. The second part of this paper comments upon the use of electron transport system (ETS) activity to assess the effect of toxicants on algae. A direct microscopic method using 2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT) for the determination of respiring viable algal cells has been developed. Respiring cells deposit INT-formazan intracellularly which can be observed by light microscopy. Algal cells that have been exposed to environmental toxicants and survived the exposure can therefore be distinguished from non-respiring cells. This allows a rapid, quantitative determination of respiring cells compared to the total number of cells in the same microscopic field.

Key words: Bioassays, toxicology, algae, sequence, electron transport activity.

TREVORS, J.T., C.I. MAYFIELD, and W.E. INNISS. 1981. Short-term toxicity tests for assessing environmental pollutants. Can. Tech. Rep. Fish. Aquat. Sci.

Des méthodes rapides d'évaluation des effets toxicologiques des produits chimiques sont nécessaires pour simplifier la tâche de tester une grande quantité de produits potentiellement toxiques. La première partie de cet article décrit une méthode qui a pour but d'étudier l'effet d'une succession d'expositions à des agents toxiques dans une évaluation biologique, employant *Pseudomonas fluorescens* comme organisme-test. Puisque les agents toxiques n'exercent pas toujours leur plein potentiel dans une application, un test a été développé en employant deux expositions successives à l'agent toxique, séparées par une période essentiellement non-existante. La réponse de l'organisme-test à différents agents toxiques est clairement reliée à la succession des additions de l'agent toxiques et à sa concentration. La deuxième partie de cet article discute de l'emploi de l'activité du système de transport d'électrons (ETS) pour évaluer l'effet des agents toxiques sur les algues. Une méthode microscopique directe employant 2-(p-iodophényl)-3-p-nitrophényl-5-

phényl chlorure de tétrazolium (INT) a été développée pour déterminer les cellules des algues vivantes qui respirent. Les cellules qui respirent déposent du formazan - INT intracellulairement qui peut être observé au microscope optique. Les cellules d'algues qui ont été exposées aux agents toxiques de l'environnement et qui ont survécu peuvent donc être différenciées des cellules qui ne respirent pas. Cette méthode permet une détermination rapide et quantitative des cellules qui respirent et de la comparer au nombre total de cellules présentes dans le champ du microscope.

INTRODUCTION

Environmental pollutants are widespread in the environment and considerable information has been compiled concerning their toxicity towards various test organisms. At the present time, short-term shake-flask and bottle tests are being proposed to assess the toxicity of pollutants to microorganisms involved in the carbon, nitrogen and sulfur cycles (Gledhill 1980). Such tests allow an estimate of the concentration of toxicant that is necessary to bring about a toxic response. In addition, since pollutants are rarely found alone in the environment, knowledge on repeated applications of single pollutants or mixtures is very important.

A number of methods have also been described for measuring the effect of pollutants on algae (APHA 1975; EPA 1978; Joubert 1980). The toxic effects of pollutants on algal species can be assessed by measuring rates of growth, photosynthesis and nutrient uptake. Also, a wide variety of microscopic algae are available for toxicity testing. However, microscopic methods do not distinguish respiring from non-respiring cells. Because respiration is closely associated with active cellular metabolism, the presence of dehydrogenase activity using INT can be used to detect respiring cells (Zimmerman et al. 1978). Such enzyme activity has been described as respiratory potential or electron transport system (ETS) activity (Jones and Simon 1979), which is an almost universal component of respiring organisms (Packard et al. 1971). Since algal cells are relatively large, the formazan deposits can be easily observed by microscopic examination. Also, algae have been extensively used in toxicity bioassays (EPA 1978) to assess environmental pollutants.

MATERIALS AND METHODS

Bioassays with Pseudomonas fluorescens

The procedure used to study the effect of sequence of exposure to pentachlorophenol (PCP) and 2,3,4,5-tetrachlorophenol (TCP) using *Pseudomonas fluorescens* ATCC 11250 as the test organism has been previously described (Trevors et al. 1981, 1982). A summary of the procedure is shown in Figure 1.

Algal Bioassays with INT

High purity grade (99%) 2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyl

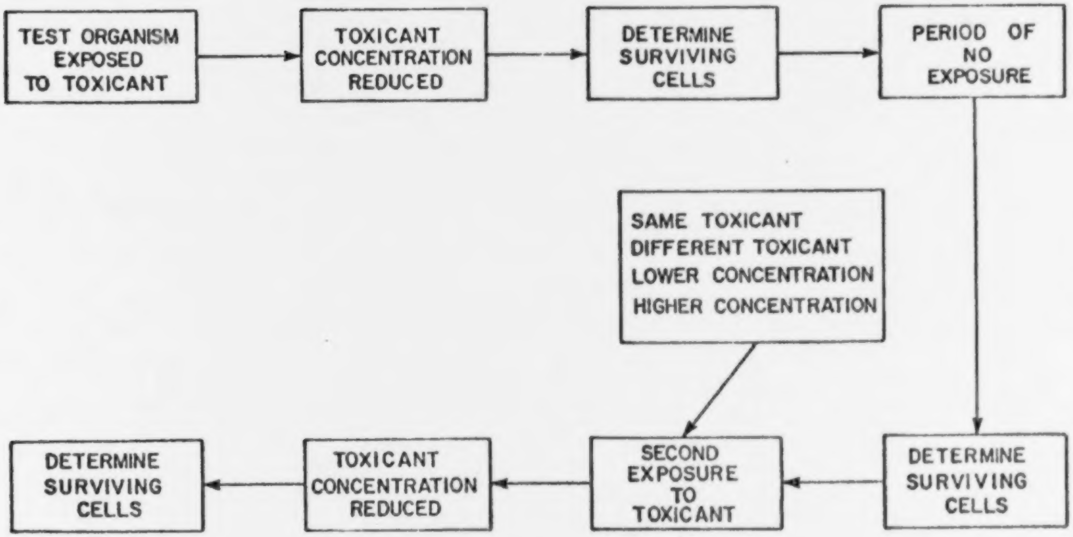

TEST ORGANISM EXPOSED TO TOXICANT
TOXICANT CONCENTRATION REDUCED
DETERMINE SURVIVING CELLS
PERIOD OF NO EXPOSURE
DETERMINE SURVIVING CELLS
SAME TOXICANT
DIFFERENT TOXICANT
LOWER CONCENTRATION
HIGHER CONCENTRATION
SECOND EXPOSURE TO TOXICANT
TOXICANT CONCENTRATION REDUCED
DETERMINE SURVIVING CELLS

Figure 1. Sequential bioassay procedure.

tetrazolium chloride was obtained from the Sigma Chemical Co., St. Louis, Mo. A 0.4% (w/v) aqueous solution of INT was filter sterilized using a 0.20 μm average pore size membrane and stored in a dark serum capped vial at 4°C.

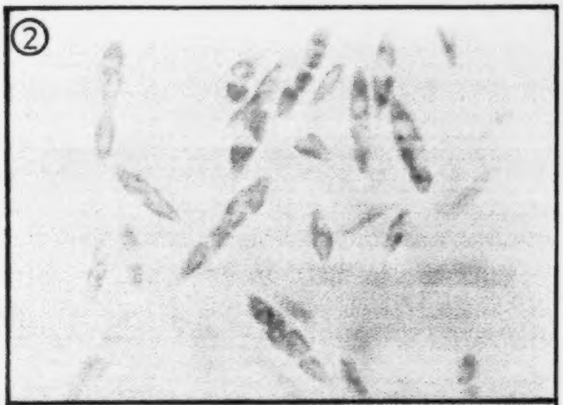
Ankistrodesmus braunii ATCC 12744, *Scenedesmus quadricauda* ATCC 11460, and *Selenastrum capricornutum* ATCC 22662 were each grown in Erlenmeyer flasks containing 17 mL of Bold basal medium (Bold and Wynne 1978) at 20°C on a rotary shaker at 120 rpm for the appropriate length of time. Fluorescent lights provided constant illumination at an intensity of 30 μEinsteins m^{-2} s^{-1}. The cells were harvested by centrifugation at 3000 x g at 4°C, washed once in sterile 150 mM phosphate buffer (pH 7) and resuspended in 17 mL of buffer to give an OD_{600} value of 0.50. All centrifuge tubes were polycarbonate and non-toxic to the algal cultures used in the present study.

The test organisms were exposed to various concentrations of mercuric chloride ($HgCl_2$, 99.3%, J.T. Baker Chemical Co., Phillipsburg, N.J.) for 24 h at 20°C. The culture flasks were incubated at 120 rpm under constant illumination. The control flasks received sterile distilled water instead of the toxicant. Mercuric chloride was prepared by dissolving the appropriate amount in sterile distilled water, and filter sterilized through a 0.20 μm pore size membrane. The toxicant was added as a 0.1 mL volume to the cell suspensions contained in Erlenmeyer flasks. At 1 h intervals, a 1.0 mL sample of the cell suspension was aseptically removed and placed in a sterile test tube. A 0.2 mL aliquot of the INT solution was added to each test tube. The tubes were gently vortexed for 10 sec and then incubated at 20°C under fluorescent lights as previously described. The reaction was allowed to proceed for 2 h, at which time the tubes were again gently vortexed for 10 sec. A 0.1-mm deep hemocytometer or a 0.2 mm deep Fuchs Rosenthal counting chamber (Stein 1973) were used to determine the total number of cells and the proportion of respiring cells. Algal cell suspensions killed by either ultraviolet light (60 min exposure 6 cm from a 10,000 μW cm^{-2} source) or by heating at 100°C for 15 min were also examined for intracellular INT-formazan deposits. Direct microscopic examination revealed no deposits within these killed cells. INT-formazan deposits were examined by transmitted bright-field illumination. The period of time required for the microscopic examination was less than 30 min. All observations were made using either an Olympus research microscope or a Nikon Labophot research microscope. A Nikon HPM camera attached to the vertical tube of the Nikon microscope was used for taking photomicrographs. The INT-formazan deposits can be enhanced during microscopic examination by using a Kodak Wratten K 12 yellow filter. A Wratten 56 green filter or a Wratten K 12 filter can be used to obtain high contrast black and white negatives for photographic records.

RESULTS AND DISCUSSION

The results of the sequential bioassay procedure using *P. fluorescens* can be summarized as follows: (1) the first exposure to a toxicant may desensitize the test organism to the second exposure of the toxicant even at a higher concentration; (2) the first exposure to the toxicant may increase the sensitivity of the test organism to the second exposure of the same or different toxicant even at a lower concentration; (3) the length of time between the

Figure 2. *A. braunii* cells containing no INT-formazan deposits.



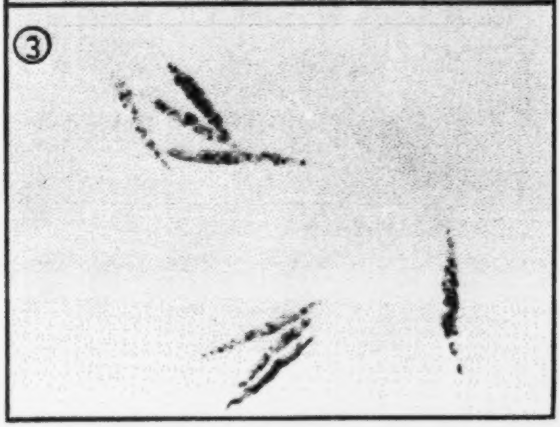


Figure 3. *A. braunii* cells containing INT-formazan deposits.

Table 1. Total cell count and respiring cell count for 8 day cell suspension of *A. braunii* treated with 1 $\mu g/mL^{-1}$ $HgCl_2$.

Incubation time (h)	Total cells ($\times 10^4$ mL^{-1})	Respiring cells ($\times 10^4$ mL^{-1})	Percent respiring
Control (no toxicant)			
0	9.5 ± 0.86	9.4 ± 0.89	98.9
1	8.4 ± 0.67	8.2 ± 0.62	97.6
2	8.6 ± 0.32	8.4 ± 0.39	97.7
3	8.5 ± 0.10	8.2 ± 0.99	96.5
4	8.0 ± 0.50	7.8 ± 0.47	97.5
5	9.5 ± 0.85	9.2 ± 0.89	96.8
6	8.4 ± 0.70	8.3 ± 0.66	98.8
24	9.8 ± 0.97	9.6 ± 0.10	98.0
Exposed to $HgCl_2$			
0	8.7 ± 0.52	8.6 ± 0.55	98.9
1	8.7 ± 0.96	7.7 ± 0.80	88.5
2	7.7 ± 0.79	6.5 ± 0.74	84.4
3	9.2 ± 0.12	6.8 ± 0.11	73.9
4	6.8 ± 0.87	4.4 ± 0.78	64.7
5	5.4 ± 0.88	3.4 ± 0.77	63.0
6	5.7 ± 0.75	2.8 ± 0.54	49.1
24	5.5 ± 0.65	1.7 ± 0.65	30.9

Mean ± S.E.M. (n=10)

first and second exposures affects the outcome of the second exposure; (4) the time interval between the first and second exposure allows for possible repair of sublethally injured cells caused by the first exposure; (5) any damage that can be repaired upon the removal of a toxicant is not as serious as damage that can not be repaired (Trevors et al. 1981, 1982).

The bioassay procedure using INT was developed to assess the effect of toxicants on selected algal species. Figure 2 shows *A. braunii* cells that contain no INT-formazan deposits. The cells shown in this figure are typical of those that were heat killed, ultraviolet light killed, or treated with $HgCl_2$. Figure 3 clearly shows the dense INT-formazan deposits that are deposited within the cells of the respiring algae. Cell suspensions of *S. quadricauda* and *S. capricornutum* were not as useful as *A. braunii* for studying ETS activity. The INT-formazan was more diffuse and not as concentrated as the dark violet deposits in *A. braunii*. *S. capricornutum* are typically smaller than *A. braunii* cells and therefore the deposits are more difficult to see during microscopic examination.

Since rapid methods are needed to assess the effects of toxicants on biological indicator organisms, INT was used to detect respiring cells while they were being exposed to a common toxicant like $HgCl_2$. An 8 day culture of *A. braunii* not treated with the toxicant displayed about 98% ETS activity during the 24 h incubation period. However, in the treatment series, the ETS activity decreased as the time of exposure to the toxicant was increased (Table 1). Thus, whereas the control series displayed no loss in the percentage of respiring cells, the cells exposed to $HgCl_2$ underwent a pronounced respiratory decrease. Therefore, the direct microscopic procedure allowed a rapid assessment of the effect of the toxicant as opposed to time-consuming methods such as measuring optical density to determine the effect on culture growth.

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THE USE OF LD50 DETERMINATION BY INTRAPERITONEAL INJECTION AS A RAPID METHOD FOR INTIAL ESTIMATES OF POLLUTANT TOXICITY TO FISH

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A useful screening procedure for assessing the hazard to fish of xenobiotic compounds is the use of quantitative structure-activity relationships. This approach requires rapid techniques for the measurement of the relative toxicity of large numbers of compounds. This paper assesses the efficacy of LD50s measured by intraperitoneal injection (IPI) relative to oral injection (OI) and aqueous exposure (AE). A very strong positive linear correlation was observed between LD50s measured by IPI and OI for chlorobenzenes and para-substituted phenols. The LD50s by IPI were slightly lower due to faster uptake into the blood, as shown by studies with radio-labelled compounds. A strong curvilinear correlation of IPI LD50s to AE LC50x was observed for para-substituted phenols. IPI represents a rapid and inexpensive method for dosing fish to toxicity tests and the results represent toxicity as measured by alternative methods.

Key words: Fish, toxicity, pollutant, LD50 determination

DIXON, D.G., P.V. HODSON, and K.L.E. KAISER. 1981. The use of LD50 determination by intraperitoneal injection as a rapid method for initial estimates of pollutant toxicity to fish. Can. Tech. Rep. Fish. Aquat. Sci.

La mesure quantitative de la relation structure-activité est un test commode pour évaluer l'effet des composés xénobiotiques sur les poissons. Cette approche nécessite des techniques rapides pour le calcul de la toxicité relative de nombreux composés. Cet article évalue l'efficacité des mesures de LD50 par injection intra-péritoneale (IPI) par rapport aux mesures de LD50 par injection buccale (OI) et exposition à l'eau (AE). Pour les chlorobenzènes et les phénols

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para-substitués, les LD50 mesurés par IPI et OI montrent un corrélation linéaire positive très élevée. Les LD50 mesurés par IPI sont un peu plus bas à cause d'une absorption plus rapide dans le sang, ainsi que démontrée par des études avec des marqueurs radioactifs. Une corrélation curvilinéaire élevée entre les LD50 mesurés par IPI et les LC50 mesurés par AE est observée pour les phénols para-substitués. IPI est une méthode rapide et bon marché pour le dosage des poissons dans les test de toxicité et les résultats représentent la toxicité telle que mesurée par différentes méthodes.

EXTENDED ABSTRACT

There is a great need for rapid methods to assess the toxic hazard to fish of an ever-increasing number of environmentally significant chemicals. One method is quantitative structure-activity correlation (QSAR), the correlation of physical and chemical properties of compounds to some measure of biological response by multiple regression techniques. Ideally, one a QSAR is established for a class of compounds, the biological activity of new members of that class can be predicted based on their chemical structure. Toxicity testing would be undertaken for those predicted to have high toxicity while the remainder would be safely ignored.

To generate an adequate biological data base for QSAR, there is a further need for rapid techniques to measure the relative toxicity to fish of large numbers of compounds. We evaluated toxicant exposure of fish by intraperitoneal injection (IPI) or by oral intubation (OI), relative to aqueous exposure. These dosing methods are more efficient than aqueous exposure since smaller amounts of pure chemical are required and the construction and maintenance of proportional diluters is unnecessary. Problems of toxicant solubility in water can be eliminated by using either water or oil carriers and there is no need for chemical determination of toxicant concentrations in exposure tanks. A further important advantage for QSAR studies of relative toxicity is the accuracy with which the "dose received" by each fish can be determined.

Triplicate LD50s were determined by IPI, using 20-30 g rainbow trout, for each of ten re-purified para-substituted phenols and twelve chlorobenzenes. Isotonic saline containing 5% ethanol was used as the carrier for phenols while cod liver oil was used for chlorobenzenes. Each LD50 involved dosing of fish with five concentrations of toxicant plus control. Ten fish were used at each concentration. The toxicant concentration in the carrier for each dose was prepared so that a 100 g fish would receive 1 mL of carrier. Each fish was anaesthetized with tricaine methane sulphonate, weighed and injected with a proportional amount of carrier based on weight. The intraperitoneal injection was on the midventral line approximately 1.5 cm posterior to the pectoral fins using a 25 gauge syringe needle and a 1.0 mL Tuberculin syringe. Care was taken to avoid piercing the gastrointestinal tract. The puncture wound was sealed with silicone grease to prevent escape of the carrier. After injection, fish were placed in holding tanks which received a continous flow of water at 15°C. Mortality was observed on a logarithmic time scale over 120 h. No mortality occurred among the 660 control fish injected with toxicant-free carrier.

Oral dosing of toxicants was used to determine LD50s for four chlorobenzenes and five phenols. With the exception of the dosing method, the design of these bioassays was identical to the above. For these tests the appropriate dose of carrier was placed into a No. 3 gelatin capsule that was placed in the stomach of the fish by intubation using a 4 mm ID glass tube.

Conventional continuous-flow aqueous-exposure techniques were used to determine triplicate 96 h LC50s for five of the phenols. The water hardness was 135 mg/L as $CaCO_3$, pH 7.6, and other characteristics are described by Hodson et al. (1980). The bioassay conditions met the criteria outlined by Sprague (1969).

All replicate LD50s and LC50s were calculated using computerized probit analysis. The values reported herein are the means of the three replicates for each chemical.

The 120 h LD50s by IPI for the chlorobenzenes ranged from 5.1 mmol/kg for 1,2,3,4-tetrachlorobenzene to 30.5 mmol/kg for 1,3,5-trichlorobenzene. The results were highly repeatable with standard deviations ranging from 2 to 9% of the means. The OI LD50s for the chlorobenzenes were consistently 22-30% higher than the IPI LD50s. There was, however, a linear correlation between the two (IPI LD50 = 0.55 + 0.76 OI LD50; $r^2 = 0.99$; $s_b = 0.02$; N = 4) that was significant at the 0.05 probability level.

The IPI LD50s for phenols were lower than those for chlorobenzenes, ranging from 0.19 mmol/kg for p-cyanophenol to 4.34 mmol/kg phenol. The results were also more variable, with standard deviations ranging from 6 to 35% of means. Once again OI LD50s were higher than IPI LD50s, by 14 to 45% and a statistically significant correlation ($P < 0.05$) between the two was evident (IPI LD50 = 0.044 + 0.781 OI LD50; $r^2 = 0.99$; $s_b = 0.04$; N = 5). The LC50s for the five phenols tested ranged from 0.0012 mM for p-(methylamino) phenol sulphate to 0.103 mM for phenol. A curvilinear relationship appears to exist between LC50s and both OI and IPI LD50s but there were insufficient data for defined correlation.

The increased toxicity of all of the chemicals by IPI, relative to OI, may be the result of a greater and more rapid mobilization of the toxicant from the IPI site. In studies with equimolar doses of ^{14}C-labelled members of both chemical sets, the toxicant concentrations in the blood reached significantly higher levels more quickly with IPI than with OI. We believe that a higher effective dose is metabolically available to the organisms and hence toxicity is increased.

The use of IPI LD50s would appear to provide a rapid and inexpensive method for determing the relative toxicities to fish of large numbers of organic contaminants to provide a suitable data base for QSAR. However, the relevance of these data to environmental exposure of fish will remain unknown until we complete studies of acute and chronic effects of aqueous exposures.

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THE EFFECT OF FLUCTUATING LEAD EXPOSURE ON LEAD UPTAKE BY RAINBOW TROUT (*SALMO GAIRDNERI*)

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HODSON, P.V., B.R. BLUNT, and S. MCGAW. 1981. The effect of fluctuating lead exposure on lead uptake by rainbow trout (*Salmo gairdneri*). Can. Tech. Rep. Fish. Aquat. Sci.

Contaminant concentrations in aquatic ecosystems vary spatially and temporally, so that surveillance data exhibit a log-normal distribution, i.e., the majority of measurements are low but a few are quite high. Contaminant concentrations in fish represent the balance between uptake and excretion. Therefore, contaminants that are taken up quickly but excreted slowly should accumulate in fish at concentrations reflecting the highest exposure concentrations rather than the means of exposure concentrations. To test this prediction, blood lead concentrations of rainbow trout were measured after 20-day exposures to waterborne lead concentrations that fluctuated about a fixed log mean. Four levels of exposure variability were used; a lead exposure characterized by wide fluctuations was expected to cause greater lead uptake by trout than the equivalent exposure with less variability.

The results were best described by the following equation: $\log_{10}$ blood lead concentration (µg/L) = 0.842 + $\log_{10}$ Waterborne lead (µg/L) + 0.02148 x Coefficient of Variation of waterborne lead. Increasing lead concentration and increasing variability of waterborne concentrations increased lead uptake by trout. Therefore, brief violations of water quality objectives may cause a greater toxicity to fish than would be expected from estimates of average exposure concentrations.

Key words: Lead, rainbow trout, lead exposure, lead uptake.

HODSON, P.V., B.R. BLUNT, and S. MCGAW. 1981. The effect of fluctuating lead exposure on lead uptake by rainbow trout (*Salmo gairdneri*). Can. Tech. Rep. Fish. Aquat. Sci.

Les concentrations de contaminants dans les écosystèmes aquatiques varient spatialement et temporairement; les données de surveillance sont distribuées selon une courbe log-normale: la plupart des données sont basses et quelques unes sont très élevées. La concentration des contaminants trouvés chez les poissons représente une balance entre l'absorption et l'excrétion. Ainsi, les contaminants absorbés rapidement et excrétés lentement devraient s'accumuler dans les poissons à une concentration reflétant la plus haute concentration plutôt que la moyenne des concentrations auxquelles ils sont exposés. Pour confirmer cette prédiction, la concentration de plomb dans le sang de truites arc-en-ciel a été mesurée après 20 jours d'exposition à des concentrations de plomb qui varient selon une moyenne de log fixe. Quatre différentes variabilités d'exposition sont employées; une exposition au plomb caractérisée par une grand variation devrait produire une plus grand absorption de plomb qu'une même exposition mais d'une plus petite variabilité.

Les résultats sont décrits par cette équation: $\log_{10}$ conc. plomb du sang ($\mu g/\ell$) = 0.842 + $\log_{10}$ conc. plomb de l'eau ($\mu g/\ell$) + 0.02148 x coefficient de variation du plomb dans l'eau. Des concentrations de plomb et des variations de concentrations élevées augmentent l'absorption du plomb par la truite. Donc, de brèves violations du niveau de qualité de l'eau peuvent causer une plus grande toxicité aux poissons que ne le prédisent les estimés de l'exposition aux concentrations moyennes.

EXTENDED ABSTRACT

Water quality objectives to protect aquatic biota from the adverse effects of contaminants are usually based on the results of acute and chronic bioassays. These bioassays measure the response of aquatic biota to continuous exposures to relatively constant concentrations of the test substances, normally distributed about an arithmetic mean. In contrast, contaminant levels in the environment fluctuated constantly and the distribution of sampling data is generally log normal (Esman and Hammad 1977). Under these conditions, one would expect that fish exposed to contaminants that are taken up quickly but excreted slowly might reflect the peaks of exposure concentrations rather than the average concentration. If this were the case, waterborne contaminants whose average concentrations were below water quality objectives might still be toxic to fish. This experiment measured the effect of fluctuating concentrations of waterborne lead on its uptake by fish.

Triplicate groups of 10 rainbow trout (*Salmo gairdneri*) each were exposed for 20 days to nominal waterborne lead concentrations of 0, 25, 50 and 100 µg/L. This exposure regime was repeated four times. In the first, waterborne lead concentrations were maintained as close as possible to the nominal concentrations (= 'constant' or control regime). In the other three exposure regimes (low, medium and high variability), the waterborne concentrations were changed daily, in a predetermined pattern, so that the $\log_{10}$ mean of each day's concentration approximated an expected or nominal concentration. The nominal variability, expressed as the Coefficient of Variation:

$$(= \text{Relative Standard Deviation(s)} = C = \frac{S}{\text{Mean}} \times 100)$$

was 6.25, 12.5 and 25%, respectively, in the low, medium and high variability regimes. The standard deviations and means were calculated after $\log_{10}$ transformations of each day's measured concentrations (IWD, 1974). The blood lead concentration of each fish was measured at the end of the 20-day exposure by the methods of Hodson et al. (1977). Simple and multiple linear regression techniques were used to compare the relationship between blood and waterborne lead concentrations at each level of variability (Steel and Torrie 1960).

Blood lead concentrations increased linearly with waterborne lead concentrations as observed in other experiments (Hodson et al. 1977). An increase of C from 3% (control variability) to 12-13% (medium variability) caused little change in blood lead concentrations. In contrast, high variability (C = 25-30%) caused a significant change in the relationship between blood and waterborne lead concentrations. Relative to the control regime, blood lead concentrations were up to five times higher in the high variability regime, for a given mean waterborne lead concentration. Assuming a critical level of blood lead associated with chronic toxicity, lead toxicity should increase with variability, i.e., a lower mean waterborne concentration will be required to elicit a given toxic effect.

The relationship between observed blood lead concentrations and the observed waterborne lead concentrations and coefficients of variation was best represented by the equation:

$$\log_{10} \text{Blood lead } (\mu g/L) = 0.82 + \log_{10} \text{Waterborne lead } (\mu g/L) + 0.02148\ C.$$

The simple linear terms were more important than the squared, cubic or interactive terms. The correlation coefficient was 0.76; i.e., the equation accounted for 76% of the variability in the observed blood lead concentrations, and this relationship was significant at the 95% confidence level.

These results have clearly demonstrated that lead uptake, and hence lead toxicity, is a function of the variability of lead exposure. Therefore, brief violations of water quality objectives may cause a greater effect on fish populations than would be expected from estimates of average exposure concentrations. By modelling expected tissue concentrations of lead from a knowledge of uptake and excretion rate constants, it should be possible to estimate the maximum integral of exposure concentration and time that will still be safe for fish. This would permit an analysis and interpretation of contaminants surveillance data that is more realistic than a simple calculation of means.

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PERSISTENCE OF PERMETHRIN, ATRAZINE AND METHOXYCHLOR IN A NATURAL LAKE SYSTEM

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J.Y. YOO and K.R. SOLOMON. 1981. Persistence of permethrin, atrazine and methoxychlor in a natural lake system. Can. Tech. Rep. Fish. Aquat. Sci.

The persistence of permethrin, atrazine and methoxychlor in (90-125 m^3) aquatic enclosures is described. Half-lives of 2-6 days for permethrin, 300 days for atrazine and 12-19 days for methoxychlor were found.

Key words: Aquatic enclosures, methoxychlor, atrazine, permethrin, persistence, pesticide persistence.

J.Y. YOO and K.R. SOLOMON. 1981. Persistence of permethrin, atrazine and methoxychlor in a natural lake system. Can. Tech. Rep. Fish. Aquat. Sci.

La persistance de la perméthrine, de l'atrazine et du méthoxychlore dans des enclos aquatiques est décrite. On a trouvé des demi-vies de 2-6 jours pour la pérmethrine, de 300 jours pour l'atrazine et de 12-19 jours pour le méthoxychlore.

EXTENDED ABSTRACT

The persistence of permethrin, atrazine and methoxychlor in an aquatic ecosystem was studied in large volume, (90-125 m^3) limnocorrals situated in a natural lake located in Southern Ontario. Techniques used in the construction, assembly, moving of limnocorrals and pesticide application methods were discussed by Solomon et al. (1980). Permethrin was applied at 5, 50, 500 $\mu g\ L^{-1}$ in 1979 and 0.5, 5 $\mu g\ L^{-1}$ in 1980, atrazine at 0.2, 2 $mg\ L^{-1}$ in 1980 and 1981 and 20 $mg\ L^{-1}$ in 1981, and methoxychlor at 3, 300 $\mu g\ L^{-1}$ in 1981. Permethrin (86.6% technical) was applied in acetone solution, atrazine (97% technical) suspended in water, and methoxychlor (240 $g\ L^{-1}$ EC) as a water emulsion. Pesticides were completely mixed with the water using a pump and injector. Water samples were collected at depths of 1, 2 and 3 m as individual samples or from surface to 3 m depth as integrated samples using a tube sampler (Solomon et al. 1982). To minimize disturbance of water, shallow samples were taken first. Water samples were stored in cold room (3-5^{o}C) and extracted within 48 hours of collecting.

Water samples (1000 mL - 1500 mL) were extracted three times for permethrin and methoxychlor with hexane (3 x 100 mL), and for atrazine with ethyl acetate (200, 100, 100 mL). Combined extracts were dried over Na_2SO_4, then quantitatively concentrated to a known volume. Permethrin and methoxychlor extracts were subjected to clean up on hexane-prerinsed Silica Gel columns (Bio-Sil A, 200-400 mesh, 3 g). Permethrin was eluted with 2.5% diethyl ether in hexane and methoxychlor, with 5% diethyl ether in hexane. The collected eluates were analyzed by GLC under the following conditions. Permethrin: Tracor 550 GLC with Ni-63 ECD, carrier and purge gas; prepurified nitrogen gas (80 mL/min. and 20 mL/min.), 1 m x 4 mm ID glass column packed with 3% OV-101 on Gas Chrom Q (100-120 mesh), injector; 260°C, oven; 260°C, detector, 300°C. Atrazine: Tracor 550 GLC with NPD (model 702), carried gas; helium (30 ml/min.), plasma gas; hydrogen (2.5 mL/min.) and air (130 mL/min.), 1 m x 4 mm ID glass column packed with 1% Carbowax 20 M on Chromosorb W HP (80-100 mesh), injector; 200°C, oven; 180°C, detector; 250°C. Methoxychlor: Varian 3700 GLC with Ni-63 ECD, carrier gas; prepurified nitrogen gas (60 mL/min.), 1.2 m x 4 mm ID glass column packed with 5% OV-101/QF-1 (2:3) on Gas Chrom Q (80-100 mesh); injector; 220°C, oven; 190°C, detector; 350°C.

Pesticide concentration in individual samples immediately after treatment differed by less than 5% indicating good mixing. Permethrin rapidly disappeared from the water (Fig. 1), the rate being almost linear in the early stage (0-15 days) after treatment when plotted on a log concentration versus time (day) basis. This suggested that a first-order kinetic rate model could be used to describe the disappearance of permethrin in water. The calculated half-life of permethrin was 2 to 4 days depending on the concentration of treatment. The rate of disappearance and average residue percentage of permethrin at 8 days after treatment with the same concentration (5 μg L^{-1}) in 1979 and 1980 was very similar (rate per day: 0.325, 0.313 and average residue percentage: 13.3, 13.9%, respectively). The average residue percentage at 8 days were 7.0, 19.3 and 23.5% for 0.5, 50 and 500 μg L^{-1} treatments, respectively. Fig. 1 shows that permethrin is not persistent in water from this lake. The rapid disappearance of permethrin from water may be due to easy adsorption to particulate matter such as suspended sediments (Sharom and Solomon, 1981 a and b) and organisms as well as rapid chemical hydrolysis.

Fig. 2 shows the persistence curves of atrazine at different concentrations in water during 1980 and 1981. Atrazine was shown to be a persistent herbicide with 42.7% remaining 418 days after treatment at 0.2 mg L^{-1}. Degradation products resulting in the removal of either the ethyl, or the isopropyl side chain, were detected from 6 days after treatment, but the results are not included in this report. Disappearance curves were similar in the two seasons with average residue levels of 66.5% in the 2 mg L^{-1} treatments and 66.8% in the 0.2 mg L^{-1} treatments at 90 days after treatment. In the very high concentration (20 mg L^{-1}), atrazine rapidly disappeared until 13 days and then followed a similar trend to the low concentrations. This fast disappearance in the early stage may be due to a rapid precipitation of insoluble atrazine.

The disappearance curves of methoxychlor in lake water show similar trends at both concentrations (Fig. 3). The average residue percentages of methoxychlor at 26 days were 24.39% and 23.03%, and 5.25% and 4.30% at 110 days, in the concentrations of 3 μg L^{-1} and 300 μg L^{-1}, respectively. The disappearance of methoxychlor from water was probably as a result of a combination of chemical, photolitic and microbiological degradation. In the high concentration the rapid drop in residues in the water immediately after treatment may have been due to precipitation. Residue levels in the sediment at 15 days after treatment were higher in 300 μg L^{-1} corrals than the 3 μg L^{-1} corrals when compared on the basis of percent applied.

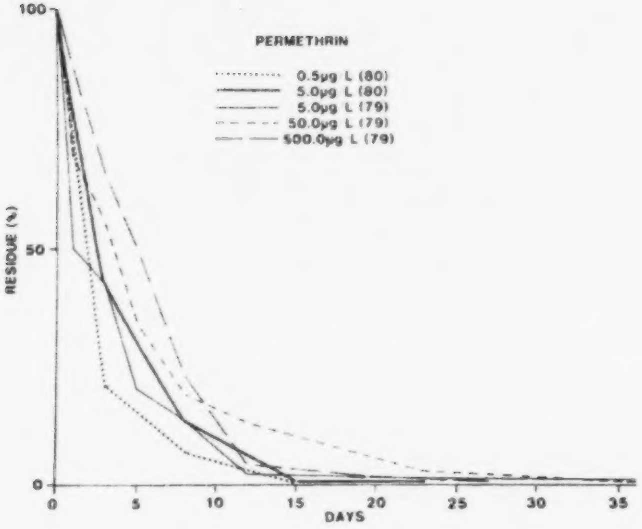

Fig. 1. Residue percentages of permethrin in natural lake water after treatment at different concentrations in 1979 and 1980. Average values of integrated samples from three replicate corrals for 1980, individual samples from three replicate corrals for 5.0 µg L^{-1} and 50.0 µg L^{-1}, and one corral for 500.0 µg L^{-1} in 1979.

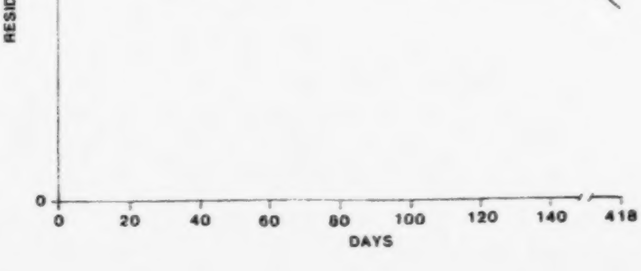

Fig. 2. Residue percentages of atrazine in natural lake water after treatment at different concentrations in 1980 and 1981. Average values of individual samples from three replicate corrals for 1980, integrated samples from three replicate corrals for 0.2 mg L^{-1} and 2.0 mg L^{-1}, and one corral for 20.0 mg L^{-1} in 1981.

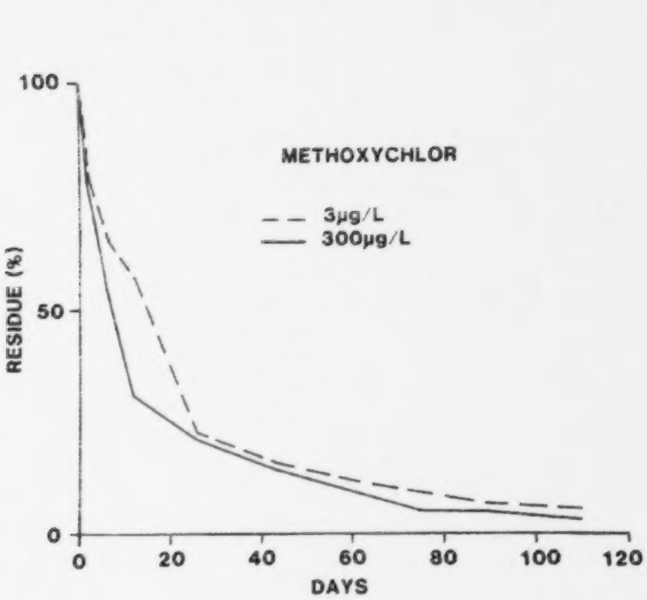

Fig. 3: Residue percentages of methoxychlor in natural lake water after treatment at different concentrations in 1981. Average values of individual samples from three replicate corrals for 300 µg L^{-1} and two replicate corrals for 3 µg L^{-1}.

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A BIOASSAY SIMULATING NATURAL EXPOSURE TO SPILLS: DILUTION EXPOSURES

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ANDERSON, J.W., S.L. KIESSER, and D.L. MCQUERRY. 1981. A bioassay simulating natural exposure to spills: Dilution exposures. Can. Tech. Rep. Fish. Aquat. Sci.

In two recent papers we have shown the usefulness of describing toxicity for crustaceans on a basis of total exposure (= concentration x time). Using a flowing exposure system, of consistent hydrocarbon component composition and concentration, we have demonstrated that the "toxicity index" (ppm-days) can be used to describe the tolerances of three crustaceans. Constant exposures to water extracts of oil at various dilutions produced 50% mortality in organisms after exposures of about 5 h to 8 days. When the time to 50% mortality is plotted on log-log scale versus concentration, the values for all 3 species produced curves of slope -1.27 and correlation coefficients between 0.8 and 0.9. Since the total exposure producing 50% mortality was consistent for each species, we next tested chemically dispersed oil in constant and diluting exposures. The toxicity index values for constant exposures to dispersed oil were very similar (based on measured hydrocarbons, ppm) to those of oil extracts. If the concept of total exposure was valid, then integration under a curve representing a diluting exposure to the test animals should produce a toxicity index equal to that of constant exposure. Indeed, the values (ppm-days) for constant exposures are equivalent to those produced in 8- and 24-h dilution exposures of the coonstripe shrimp, *Pandalus danae*. An exposure system has been constructed which will linearly dilute chemically dispersed oil to zero in either 8 or 24 h. Data have been produced, over about one year, which demonstrate the consistency of the method for testing toxicity, the alteration in tolerance in the shrimp with season and allow prediction of effects from realistic spill situations.

ANDERSON, J.W., S.L. KIESSER, and D.L. MCQUERRY. 1981. A bioassay simulating natural exposure to spills: Dilution exposures. Can. Tech. Rep. Fish. Aquat. Sci.

Deux récents ouvrages ont montré l'utilité d'exprimer la toxicité pour les crustacés en terme d'exposition totale (= concentration x temps). En employant un système d'exposition à l'eau courante dont la composition et la concentration d'hydrates de carbone sont consistantes, on a démontré que "l'index de toxicité" (ppm - jours) peut être utilisé pour décrire les niveaux de tolérance de 3 différents crustacés. Une exposition constante des crustacés à de l'eau extraite d'huile, à différentes dilutions, cause la mort de 50% des organismes après des expositions variant de 5 heures à 8 jours. Si on établit

une courbe de la période de temps qui produit une mortalité de 50% sur une échelle log-log en fonction de la concentration, les 3 espèces ont une courbe dont la pente est -1.27 et des coefficients de corrélation se situant entre 0.8 et 0.9. Puisque l'exposition totale qui produit une mortalité de 50% est consistante pour chaque espèce, on a ensuite testé les huiles dispersées chimiquement dans des expositions constantes et diluées. Les valeurs de l'index de toxicité pour des expositions constantes à l'huile dispersée sont très similaires (basé sur les mesures de concentration des hydrocarbones en ppm) à celles des extraits d'huile. Si le concept de l'exposition totale est valable, l'intégrale sous une courbe qui représente les expositions diluées devrait produire un index de toxicité égal à celui de l'exposition constante. En effet, les valeurs (ppm - jours) dans le cas d'expositions constantes sont l'equivalent de celles produites par expositions diluées de 8 et 24 heures de la crevette, *Pandalus danae*. Un système d'exposition a été construit et il peut diluer linéairement l'huile dispersée chimiquement à 0 en un temps de 8 ou 24 heures. Des résultats, produits depuis plus d'un an, démontrent la consistance de cette méthode pour tester la toxicité, le changement du niveau de tolérance chez les crevettes avec les saisons et permet de prédire les effets des déversements de nappes d'huile.

ABSENCE OF ACCLIMATION TO PARATHION BY RAINBOW TROUT

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BANAS, W.P. and J.B. SPRAGUE. 1981. Absence of acclimation to parathion by rainbow trout. Can. Tech. Rep. Fish. Aquat. Sci.

Acclimation by fish to environmental factors such as temperature and dissolved oxygen has been well documented. Recent studies have shown that fish can also acclimate to toxicants (copper and arsenic). The objective of this study was to determine whether rainbow trout, *Salmo gairdneri*, would acclimate to parathion, an organophosphate insecticide which represents another class of pollutants, having a different mode of action than those toxicants in previous acclimation studies.

Rainbow trout did not change their tolerance to lethal levels of parathion after 0, 1, 2, 3 and 5 weeks of pre-exposure to 0.31 mg/L. Both pre-exposed and control groups maintained an incipient LC_{50} of 1.26 mg/L. The pre-exposure concentration represents 0.26 of the control incipient LC_{50} obtained at 15°C. Thresholds of mortality were reached at 120-144 h for all bioassays, though parathion pre-exposed fish consistently began to die earlier than controls.

Both groups grew throughout the experiment and there was no difference in dry weight after one week. The dry weight of parathion-treated fish was significantly reduced by a factor of 1.2 (2 and 3 week exposure) and 1.3 (5 week exposure). Parathion-treated fish also exhibited less activity than controls. Contributing factors such as loss of appetite and the transfer of resources utilized in growth towards increased detoxification capabilities may explain the growth reduction of parathion-treated fish.

The length of the pre-exposure period represents the maximum time fish could be exposed to parathion in the environment due to its lack of persistence. This study further demonstrates that fish have a different acclimation response to various toxicants.

BANAS, W.P. and J.B. SPRAGUE. 1981. Absence of acclimation to parathion by rainbow trout. Can. Tech. Rep. Fish. Aquat. Sci.

L'acclimatement des poissons aux facteurs environnementaux tels que température et quantité d'oxygène dissout est bien documenté. Des ouvrages récents montrent que le poisson peut aussi s'acclimater aux substances toxiques (cuivre et arsenic). L'objectif de cette étude est de déterminer si la truite arc-en-ciel, *Salmo gairdneri*, s'adapterait ou non au parathion,

un insecticide organophosphate qui représente une autre classe de polluants ayant un mode d'action différent de celui des nombreuses autres substances toxiques mentionnées dans des études antérieures sur l'acclimatement.

La truite arc-en-ciel ne change pas sa tolérance face au niveau léthal de parathion après 0,1,2,3 et 5 semaines de pré-exposition à une concentration de 0.31 mg/ℓ. Les deux groupes concernés, c'est-à-dire groupes pré-exposés et groupes de contrôle maintiennent un CL 50 initial de 1.26 mg/ℓ. La concentration de pré-exposition représente 0.26 du CL 50 du contrôle à 15°C. Les seuils de mortalité sont atteints après 120-144 h pour l'ensemble des essais biologiques, mais les poissons pré-exposés au parathion meurent de façon consistante avant ceux utilisés comme contrôle.

Les deux groupes ont augmenté de poids durant l'expérience. On ne constate aucune différence dans le poids sec après une semaine. Le poids sec des poissons traités au parathion est réduit d'une façon significative par un facteur de 1.2 (2 et 3 semaines d'exposition) et 1.3 (5 semaines d'expositions). Les poissons traités au parathion sont aussi moins actifs que ceux de contrôle. Des facteurs tels que perte d'appétit et transfer des ressources utilisées pour la croissance dans le but d'augmenter les capacités de détoxification peuvent expliquer le ralentissement dans la croissance des poissons traités au parathion.

La durée de la période de pré-exposition représente le temps maximum pendant lequel le poisson pourrait être exposé au parathion dans l'environnement, dû à son manque de persistance. Cette étude démontre de plus que les poissons ont différentes réponses d'acclimatement pour différentes substances toxiques.

THE INFLUENCE OF PH, HARDNESS AND ALKALINITY ON THE ACUTE TOXICITY OF ZINC TO RAINBOW TROUT

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BRADLEY, R.W. and J.B. SPRAGUE. 1981. The influence of pH, hardness and alkalinity on the acute toxicity of zinc to rainbow trout. Can. Tech. Rep. Fish. Aquat. Sci.

Dissolved zinc was much less toxic to trout in hard than in soft water (31 and 386 mg/L $CaCO_3$), and in acid water (pH 5.5) than in neutral (pH 7.0) or alkaline water (pH 9.0).

The increase in hardness from 31 to 386 mg/L reduced total zinc toxicity by more than an order of magnitude at all pH levels. At pH 5.5 and alkalinity <1 mg/L $CaCO_3$, the LC_{50} was increased from 0.88 to 10.5 mg/L. At pH 7.0, and alkalinity 8.3 mg/L, the total zinc LC_{50} increased from 0.17 to 4.46 mg/L. When the alkalinity was increased from 8.3 to 24 mg/L, the LC_{50} at pH 7.0 increased from 0.19 mg/L at low hardness to 5.16 mg/L zinc at high hardness. At pH 9.0, and alkalinity 23 mg/L, the LC_{50} increased from 4.5 to >88 mg/L total zinc.

The decrease in zinc toxicity at high hardness was due to hardness alone, since the pH and alkalinity were held constant. The increase in alkalinity from 8.3 to 24 mg/L at pH 7.0 did not significantly alter zinc toxicity. Since higher alkalinity levels could not be maintained at this pH, it is concluded that alkalinity is not an important factor at or below pH 7.

The reduction in zinc toxicity at pH 5.5, compared to pH 7.0, was significant at both hardness levels; at low hardness, the LC_{50} was decreased by a factor of 5 (0.88 to about 0.18 mg/L zinc), and at high hardness by a factor of 2 (10.5 to about 4.8 mg/L zinc). This change in toxicity did not result from a change in the chemical speciation of zinc. At both pH levels all zinc was in solution, and over 90% was present as the aquo zinc ion, $(Zn[H_2O]_6)^{2+}$, according to: a) total zinc measurements on filtered and unfiltered water; b) measurements by differential pulse polarography; and c) predictions from a computer model for chemical equilibria.

It is clear that zinc precipitates are of low toxicity. At pH 9.0 and high hardness, no fish mortality occurred at a total zinc concentration of 88 mg/L. In this test, all but 0.3% (0.03 mg/L) of the zinc was present as a precipitate $(Zn[OH]_x)$.

At pH 9.0 and low hardness, fish mortality did occur, and must be attributed in part to the zinc precipitate. In this test, about 0.05 mg/L zinc was in solution; the anticipated LC_{50} was about 0.02 mg/L zinc, based on low-hardness results at pH 5.5 and 7.0. While there appears to have been enough soluble zinc present to cause mortality, the increased mortality (10%, 30%, and 80%) seen in the 3 highest test concentrations must have been caused

by the increased levels of zinc precipitate, since soluble zinc levels were similar in each tank.

The long-standing question about the influence of pH on zinc toxicity is therefore resolved on the basis of the two competing mechanisms which occur with increasing pH. Soluble zinc becomes more toxic at higher pH, but is reduced in concentration and replaced by the slightly toxic zinc precipitate. At higher pH and hardness levels, the low solubility of zinc can prevent mortality.

BRADLEY, R.W. and J.B. SPRAGUE. 1981. The influence of pH, hardness and alkalinity on the acute toxicity of zinc to rainbow trout. Can. Tech. Rep. Fish. Aquat. Sci.

La présence du zinc sous forme dissoute est beaucoup moins toxique pour la truite dans l'eau dure que dans l'eau douce (31 et 386 mg/ℓ $CaCO_3$), de même que dans l'eau acide (pH 5.5) par rapport à l'eau neutre (pH 7.0) ou l'eau alcaline (pH 9.0).

Une augmentation de 31 à 386 mg/ℓ dans la dureté de l'eau réduit la toxicité totale du zinc par plus d'un ordre de grandeur pour chaque valeur de pH. Au pH 5.5 et à une alcalinité < 1 mg/ℓ $CaCO_3$, le LC_{50} passe de 0.88 a 10.5 mg/ℓ. Au pH 7.0 et à une alcalinité de 8.3 mg/ℓ, le LC_{50} du zinc total passe de 0.17 à 4.46 mg/ℓ. Avec une élévation du niveau d'alcalinité de 8.3 à 24 mg/ℓ, le LC_{50} à pH 7.0 passe de 0.19 mg/ℓ (faible dureté) à 5.16 mg/ℓ zinc (forte dureté). Au pH 9.0 et à un taux d'alcanité de 23 mg/ℓ, le LC_{50} passe de 4.5 à > 88 mg/ℓ en zinc total.

Une baisse dans la toxicité du zinc pour une dureté élevée s'explique seulement par la dureté, le pH et l'alcalinité étant tenus constants. Une élévation de 8.3 mg/ℓ à 24 mg/ℓ du taux d'alcalinité à pH 7.0 ne produit pas de changement significatif au niveau de la toxicité du zinc. Le fait de ne pouvoir maintenir un taux d'alcalinité plus haut à ce pH laisse à conclure que l'alcalinité n'est pas un facteur à considérer à un pH inférieur ou égal à 7.

Au pH 5.5, une diminution dans la toxicité du zinc, comparativement à celle observée au pH 7.0, est significative aux deux niveaux de dureté. A faible dureté, le LC_{50} diminue par un facteur de 5 (de 0.88 à environ 0.18 mg/ℓ zinc) alors qu'à dureté élevée, le facteur de variation est de 2 (10.5 à environ 4.8 mg/ℓ zinc). Ce changement dans le degré de toxicité n'est pas attribué à un changement de l'état chimique du zinc. Pour ces deux différents pH, on trouve que tout le zinc est en solution et que plus de 90% est présent sous forme d'ion zinc hydraté, $(Zn\,[H_2O]_6)^{2+}$. Ces informations reposent sur: a) des mesures de la quantité totale de zinc dans l'eau filtrée et non-filtrée; b) des mesures par polarographie à pulsation différentielle; c) des prédictions à partir d'un modèle d'équilibre chimique fait par ordinateur.

Il est clair que les précipités de zinc ont une faible toxicité. Un pH 9.0 et une dureté élevée ne produisent pas de mortalité chez le poisson à une concentration totale de zinc égale à 88 mg/ℓ. Dans ce test, tout le zinc, exception faite de 0.3% (0.03 mg/ℓ), est présent sous forme de précipité ($Zn [OH]_x$).

Au pH 9 et à faible dureté, il y a mortalité de poissons. Ceci doit, en partie, être attribué à la présence du précipité de zinc. Dans ce test, environ 0.05 mg/ℓ de zinc est en solution. Le LC_{50} anticipé est d'environ 0.02 mg/ℓ zinc, basé sur les résultats obtenus avec une faible dureté, aux pH 5.5 et 7.0. Bien qu'il semble qu'il y ait assez de zinc soluble pour causer la mortalité, une élévation du degré de mortalité (10%, 30% et 80%) observée lors des tests impliquant les 3 plus hautes concentrations doit avoir été causée par une augmentation de la quantité de précipité de zinc, puisque le niveaux de zinc solubles sont similaires dans chacun des réservoirs.

La question de l'influence du pH sur la toxicité du zinc est donc résolue, basée sur deux mécanismes compétitifs, apparaissant avec une élévation de pH. Le zinc soluble devient plus toxique à pH plus élevé, mais il diminue en concentration et est remplacé par un précipité de zinc légèrement toxique. Aux pH et niveaux de dureté plus élevés, une faible solubilité du zinc peut empêcher la mortalité.

FATE OF ^{14}C-1,3,6,8-TETRACHLORODIBENZO-P-DIOXIN (1,3,6,8-TCDD) IN A MODEL POND SYSTEM: PRELIMINARY RESULTS

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CORBET, R.L. and G.R.B. WEBSTER. 1981. Fate of ^{14}C-1,3,6,8-Tetrachlorodibenzo-p-dioxin (1,3,6,8-TCDD) in a model pond system: Preliminary results. Can. Tech. Rep. Fish. Aquat. Sci.

The manufacture of 2,4,5 trichlorophenol, by alkaline hydrolysis of 1, 2,4,5 tetrachlorobenzene leads to the formation of dioxin by-products. The major dioxin formed in this case is the 2,3,7,8-isomer, the most toxic dioxin and among the most toxic compounds known to man. Manufacture of the herbicide 2,4-D involves the prior synthesis of 2,4-dichlorophenol by the chlorination of phenol. Although dioxin by-products had not been expected, Cochrane et al. (1981) reported the presence of several di-, tri-, and tetra-chloro dioxin contaminants in samples of technical grade 2,4-D esters and amines with the most prominent isomer being 1,3,6,8-tetrachlorodibenzo-p-dioxin. The heavy use of 2,4-D herbicides in agriculture and the chemical stability and persistence of dioxins make the fate of these compounds a unique problem in environmental chemistry. Since 2,4-D is applied directly to aquatic systems for weed control and since runoff and drift carry residues of aerially supplied 2,4-D to aquatic systems, studies using replicated model pond systems were initiated in 1981.

Four ponds were designed and built at the Glenlea Research station near Winnipeg. Each pool measured 4.0 m by 5.5 m with a 0.5 m depth (volume: 5200 L). The pools were lined with 10 mil. polyethylene plastic and clay base sod with the bottom overlaid with layers of sand and soil. Each pool was equipped with a self leveling water system with an external reservoir. Three of the pools were injected with one of three concentrations of universally labelled ^{14}C-1,3,6,8-TCDD (100, 250 and 1000 ppt) while the fourth was maintained as a control. Samples were taken of three species of invertebrates, sediment, water, vegetation (aquatic), fish and the air above the pools for a period of 90 days.

The water samples were extracted with dichloromethane and counted directly by a LS7500 Beckman liquid scintillation counter. The air was sampled using a three polyurethane foam stratified sampler drawing 8.0 L/min of air per foam. The foams were extracted for 2 to 3 h with hexane by soxhlet extractor and extracts analyzed by liquid scintillation counting. The soil and invertebrates were combusted in a Packard Oxidizer, the activity counted and, as above, converted to mg of TCDD by multiplication by the specific activity.

The preliminary results indicate that the labelled material moves through the water column (90% within 96 h) and into the air above the pool. This was not totally unexpected due to the non-polar nature of the compound and its low estimated solubility (200 ng/L). Similarly the activity in the sediment increased over the first week of the study. A clearance experiment with one

species (*Gyraulus* sp.) indicated no clearance of label occurred. It was also noted that at the higher concentration, the level of accumulation differed between the pelagic and benthic dwellers. The snails accumulated labelled material at rates 3000 to 5000 times the concentration in the surrounding media. Further information is forthcoming as the vegetation, invertebrate and fish materials are analysed.

CORBET, R.L. and G.R.B. WEBSTER. 1981. Fate of ^{14}C-1,3,6,8-Tetrachlorodibenzo-p-dioxin (1,3,6,8-TCDD) in a model pond system: Preliminary results. Can. Tech. Rep. Fish. Aquat. Sci.

La fabrication du 2,4,5 trichlorophénol par hydrolyse alcaline du 1,2,4, 5 tétrachlorodibenzène conduit à la formation de sous-produits de la dioxine. La dioxine majeure formée dans ce cas est l'isomère 2,3,7,8 considéré comme étant la dioxine la plus toxique et parmi les composés les plus toxiques connus par l'homme. La fabrication de l'herbicide 2,4-D consiste, en un premier temps, en la synthèse du 2,4-dichlorophénol par la chlorination du phénol. En dépit du fait que l'on ne s'attend pas à l'apparition de sous-produits de dioxine, Cochrane et al. (1981) ont reporté la présence de plusieurs contaminants: di- , tri- , et tétra-chloro - dioxine dans les échantillons de 2,4-D ester et d'amines (de qualité technique), l'isomère prédominant étant le 1,3,6,8-tétrachlorodibenzo-p-dioxine. L'usage répandu des herbicides de 2,4-D en agriculture ainsi que la stabilité chimique et la persistance des dioxines font du sort de ces composés un problème unique en chimie de l'environnement. Puisque le 2,4-D est appliqué directement sur les systèmes aquatiques pour le contrôle des malherbes, puisque l'écoulement d'eau des terres et l'entraînement ou charriage transporte des résidus de 2,4-D fournis aux systèmes aquatiques par voie aerienne, des études employant des modèles de simulation ont débuté en 1981.

Quatre bassins ont été dessinés et construits à la station de Recherche Glenlea, à proximité de Winnipeg. Chacun d'eux mesure 4.0 m par 5.5 m avec une profondeur de 0.5 m (Volume: 5,200 ℓ). Chaque bassin est doublé de plastique de polyéthylène (10 mil.), avec de l'argile à la base et le fond est recouvert de couches de sable et de terre. Chaque bassin comprend un système régulateur d'eau autonome ainsi qu'un réservoir extérieur. Trois des quatre bassins ont reçu une dose spécifique de ^{14}C-1,3,6,8-TCDD (100, 250 et 1000 ppt), alors le quatrième est un contrôle. On a prélevé des échantillons de trois espèces d'invertébrés, de sédiment, d'eau, de végétation aquatique, de poissons et d'air au-dessus des bassins pendant 90 jours.

On a extrait le échantillons d'eau avec du dichlorométhane et on a procédé au comptage directement avec un compteur à scintillation liquide LS7500 Beckman. On a utilisé pour l'air un appareil formé de 3 couches de mousse de polyuréthane stratifiées, aspirant 8.0 ℓ/min d'air par niveau de mousse. Les couches de mousse ont été extraites à l'hexane pendant 2 à 3 h par un extracteur soxhlet. Les extraits ont été analysés par comptage à scintillation liquide. La terre et les invertébrés ont subi un traitement de combustion dans un appareil à oxydation Packard, leur activité a été comptée et transformée en mg de TCDD en multipliant par l'activité spécifique.

Les résultats préliminaires indiquent que le matériel étiquetté par radio-activité se déplace à travers la colonne d'eau (90% à l'intérieur d'une période de 96 h) et dans l'air au-dessus du bassin. Ce phénomène n'est pas complètement inattendu étant donnè la nature non-polaire et la faible solubilité (200 ng/ℓ) du composé. De même, l'activité dans les sédiments augmente au cours de la première semaine d'étude. Une expérience avec une espèce (*Gyraulus* sp.) indique qu'aucun matériel radioactif n'est dégagé dans l'environnement. Il est noté, également, qu'à forte concentration, le niveau d'accumulation diffère entre les occupants des zone pélagique et benthique. L'accumulation de la matière radioactive par les escargots est 3000 à 5000 fois plus grande que sa concentration dans l'environnement immédiat. D'autres informations s'ajoutent à mesure que les experiences sur la végétation, les invertébrés et les poissons sont analysés.

THE BEHAVIOR OF THE AMPHIPOD *GAMMARUS LACUSTRIS* EXPOSED TO VARIOUS PH VALUES AND COPPER CONCENTRATIONS IN A COUNTER CURRENT TROUGH

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DE MARCH, B.G.E. 1981. The behaviour of the amphipod *Gammarus lacustris* exposed to various pH values and copper concentrations in a counter current trough. Can. Tech. Rep. Fish. Aquat. Sci.

To demonstrate behavioral effects of copper on the freshwater amphipod *Gammarus lacustris*, experiments were designed to demonstrate the effects of chemical factors normally accompanying copper additions, as well as the effects of copper. The first experiment examined the effects of only pH and dilution with distilled water; the second, the effects of copper accompanied by unavoidable changes in pH and dilution. It was concluded that *G. lacustris* avoided reduced osmotic pressure and reduced pH, preferred increased pH, moved less consistently at increased pH, avoided low copper levels between 10^{-6} to 10^{-11} mg/L (Cu^{++}) (calculated from equilibrium constants), preferred higher copper concentrations, and slowed down at the lower copper levels. The variety of behavioral responses described in the literature could be due to simultaneous changes in pH, dilution, and copper, and to different experimental design parameters allowing different factors to be effective first. Results confirm the belief that sensory enhancement takes place at high copper concentration, and depression at low concentrations.

Results were more consistent when presented in terms of pH and (Cu^{++}), rather than pH and total dissolved copper. The effects of pH and (Cu^{++}) may be independent and additive, whereas the effects of pH and total copper must be described interactively. The results suggest that one of the copper hydroxide species may have affected the results, but its effect is not clear.

DE MARCH, B.G.E. 1981. The behaviour of the amphipod *Gammarus lacustris* exposed to various pH values and copper concentrations in a counter current trough. Can. Tech. Rep. Fish. Aquat. Sci.

Pour démontrer les effets du cuivre sur le comportement de l'amphipode d'eau fraîche, *Gammarus lacustris*, des expériences furent planifiées afin de démontrer les effets des facteurs chimiques qui accompagnent normalement les additions de cuivre, aussi bien que les effets du cuivre lui-même. La première expérience examine les effets du pH et de dilution dans l'eau distillée; la seconde, les effets du cuivre accompagnés par des changements inévitables du degré de dilution et du niveau de pH. Il a été conclu que *Gammarus lacustris* évite les conditions de faible pression osmotique et de faible pH, préfère un pH élevé, se déplace moins constamment à ce même pH, évite de faibles concentrations en cuivre, entre 10^{-6} et 10^{-11} mg/ℓ (Cu^{++}) (calculé à partir des constantes d'équilibre), préfère de plus fortes concentrations en cuivre et ralentit à des niveaux inférieurs de cuivre.

La variété dans les réponses de comportement décrites dans la littérature peut être due aux changements simultanés de pH, de dilution et de cuivre ainsi qu'aux différents paramètres du design expérimental qui permettent à différents facteurs de faire effet en premier. Les résultats confirment l'idée que des améliorations sensorielles prennent place à des hautes concentrations en cuivre alors que surviennent des diminutions à faibles concentrations.

Les résultats sont plus consistants lorsque présentés en termes de pH et (Cu^{++}) au lieu de pH et concentration totale de cuivre dissous. Les effets du pH et de (Cu^{++}) peuvent être à la fois indépendants et additifs, alors que les effets du pH et du cuivre total doivent être décrits de façon interactive. Les résultats suggèrent qu'une des formulations d'hydroxyde de cuivre peut avoir affecté les résultats, mais cet effet n'est pas clair.

RAINBOW TROUT CELLS IN CULTURE AS A MODEL FOR THE EVALUATION OF THE TOXICITY OF AQUATIC POLLUTANTS

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DENIZEAU, F. and M. MARION. 1981. Rainbow trout cells in culture as a model for the evaluation of the toxicity of aquatic pollutants. Can. Tech. Rep. Fish. Aquat. Sci.

Rainbow trout cells in culture were used to develop a new bioassay for the evaluation of sub-acute effects of water pollutants. The cell line RTG-2 derived from the gonads was chosen. The cells were grown at 15°C in 60 mm petri dishes in modified Eagle's Minimal Essential Medium (MEM) supplemented with 10% foetal bovine serum (FBS). Under these conditions, an inoculum of 10^5 cells produced within 18 days a continuous monolayer with a maximum cell number of approximately 2×10^6. In order to follow the growth of the cells, we proceeded to the determination of the total protein, DNA and RNA content. Simultaneously, the incorporation of (^{3}H) thymidine in DNA and (^{14}C) uridine in RNA over a 48 h period was measured. The effect of lead (Pb), cadmium (Cd) and polychlorinated biphenyls (PCB) on these five parameters was investigated.

The toxicity studies were carried out in the following manner: two days after the inoculation (10^5 cells) when most cells have attached to the substratum, the growth medium was aspirated and fresh medium containing the pollutant (except for controls) was added. Thereafter, the medium was changed every two days until the experiment was terminated with the appearance of confluency in the controls. This allowed the possibility of exposure of at least four generations of cells to the toxic agent. Immediately after the medium was changed, 1 µCi of (^{3}H) thymidine and 1 µCi of (^{14}C) uridine were added in some samples. Forty-eight hours later, the proteins, DNA and RNA were extracted from the samples that had received the radioactivity. The proteins were measured by the method of Bradford; the DNA and RNA concentrations were obtained by the absorbance of 260 nm. The amount of (^{3}H) in DNA and (^{14}C) in RNA was determined by scintillation counting.

At a concentration of 2.4 ppm, lead almost completely inhibited the growth of the cells. Under these conditions, the level of proteins, DNA and RNA increased slightly with time, whereas in corresponding controls, this level increased at least ten times in 18 days. The incorporation of labelled nucleotides was also dramatically lower in the treated samples. This inhibition appeared as early as 4 days after the addition of the metal and it persisted for the rest of the growth period. At 100 ppb of Pb, such effects could not be detected. A slight but not significant decrease in the total protein and nucleic acid content was observed at the end of the growth period.

In contrast to the observations with lead, the detection of cadmium toxicity depended upon the concentration of FBS in the medium. At 2.4 ppm of Cd and 10% of FBS, the protein and nucleic acid levels as well as the incorporation of radioactivity remained unaffected. However, when the concentration of FBS was reduced to 1%, the toxicity of Cd at 2.4 ppm was comparable to that of lead at the same concentration.

The sensitivity of rainbow trout cells with respect to the PCB was studied using Aroclor 1254. The minimum PCB concentration which resulted in measurable effects within the period of time required to reach confluency was 1 ppm. After 16 days of exposure at this concentration, a 60% decrease in the incorporation of (^{3}H) in DNA was observed while the total protein and nucleic acid content exhibited no difference.

The results obtained so far with rainbow trout cells in culture point to the potential of the model that these cells offer for the evaluation of subacute toxic effects of water pollutants.

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DENIZEAU, F. and M. MARION. 1981. Rainbow trout cells in culture as a model for the evaluation of the toxicity of aquatic pollutants. Can. Tech. Rep. Fish. Aquat. Sci.

Des cellules de truites arc-en-ciel en culture ont été utilisées pour développer un nouveau test biologique pour évaluer les effets sous-critiques des polluants de l'eau. La lignée de cellules RTG-2 dérivée des gonades a été choisie. Les cellules furent cultivées à 15°C dans le milieu essentiel minimal modifié de Eagle supplémenté de sérum de bovin foetal à 10% (FBS). Sous ces conditions, un inocolum de 10^5 cellules produisent en dedans de 18 jours une monocouche continue renfermant un nombre maximal approximatif de 2×10^6 cellules. Afin de suivre la croissance des cellules, on a déterminé la quantité de protéines, d'ADN et d'ARN présents. Simultanément, l'incorporation de (^{3}H) thymidine dans l'ADN et de (^{14}C) uridine dans l'ARN pour une période de 48 heures ont été mesurées. Les effets du plomb (Pb), du cadmium (Cd) et de biphényles polychlorés (PCB) sur ces cinq paramètres furent également sujets à investigation.

Des études de toxicité ont été exécutées de la façon suivante: deux jours après l'innoculation (10^5 cellules), alors que la plupart des cellules se sont attachées au substrat, le milieu de culture fut aspiré et du milieu contenant le polluant fut ajouté (sauf pour les contrôles). De plus, le milieu a été changé chaque 2 jours jusqu'à ce que l'expérience soit terminée avec l'apparition de confluence dans les contrôles. Ceci a permis d'exposer à l'agent toxique au moins quatre générations de cellules. Immédiatement après le changement du milieu, 1 µCi de (^{3}H) thymidine et 1 µCi de (^{14}C) uridine ont été ajoutés à quelques échantillons. Quarante-huit heures plus tard, les protéines, l'ADN et l'ARN furent extraites des échantillons soumis à la radioactivité. Les protéines furent mesurées par la méthode de Bradford; les concentrations en ADN et ARN, par absorption à une longueur d'onde de 260 nm. La quantité de (^{3}H) dans l'ADN et de ^{14}C) dans l'ARN furent déterminée par comptage par scintillation.

A une concentration de 2.4 ppm, le plomb empêche presque complètement la croissance des cellules. Sous ces conditions, le niveau des protéines, d'ADN et d'ARN augmente légèrement avec le temps, alors que dans les contrôles correspondants, ce niveau augmente d'au moins dix fois en 18 jours. L'incorporation de nucléotides étiquettées par radio-activité diminue dramatiquement dans les échantillons traitées. Cette inhibition se manifeste aussi tôt que 4 jours après l'addition de métaux et persiste jusqu'à la fin de la période de croissance. A une concentration de 100 ppb de Pb, de tels effets n'ont pu être détectés. Une légère diminution non significative dans la quantité totale de protéines et d'acides nucléiques est observée à la fin de la période de croissance.

Contrairement aux observations faites avec le plomb, la détection de la toxicité du cadmium est liée à la présence de FBS dans le milieu. A 2.4 ppm de Cd et 10% de FBS, les niveaux de protéines et d'acides nucléiques ainsi que l'incorporation de radioactivité ne semblent pas avoir été affectés. Cependant, avec une diminution de la concentration de FBS à 1%, la toxicité du Cd à 2.4 ppm est comparable à celle du plomb à la même concentration.

La sensibilité des cellules de la truite arc-en-ciel au PCB est étudiée par l'intermédiaire du Aroclor 1254. La concentration minimale de PCB, qui résulte dans des effets mesurables à l'intérieur de la période de temps nécessaire pour atteindre la confluence, est de 1 ppm. Apres 16 jours d'exposition à cette concentration, une diminution de 60% dans l'incorporation de (^{3}H) dans l'ADN est observée alors que la quantité totale de protéines et d'acides nucléiques ne montre aucune différence.

Les résultats obtenus dans l'étude des cellules en culture de la truite arc-en-ciel jusqu'à maintenant montrent le potentiel du modèle que ces cellules offrent pour évaluer les effets toxiques sous-critiques des polluants de l'eau.

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ESTIMATING THE RATE OF LAKE ACIDIFICATION USING PH INDICATOR DIATOMS

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DICKMAN, M., J. FORTESCUE, M. OUELLET, J. TERASMAE, and I. THOMSON. 1981. Estimating the rate of lake acidification using pH indicator diatoms. Can. Tech. Rep. Fish. Aquat. Sci.

The diatom inferred pH and the observed lake pH were compared for 27 Canadian study lakes north of Lake Superior. The planktonic diatom inferred pH was within half a pH unit of the observed pH in 13 of the 27 lakes (48%) and within one pH unit in 20 (74%) of the study lakes. By examining sediment cores from three of the 27 study lakes we inferred the lake's pH at successive depths (i.e. time) based (as above) on the diatom species composition and relative abundance. The rate of change in lake pH was then estimated by comparing the diatom inferred pH in shallow and deep portions of the core. The Cesium 137 horizon and the *Ambrosia* pollen horizon were used to estimate lake deposition rates and sediment core ages. Using Husted's autecological studies of diatoms as pH indicators it was possible to conclude that Lake B, a humic lake north of Lake Superior, had not undergone rapid acidification during the last 40 years. Lake F, a clear water circumneutral lake had undergone a reduction in pH from 8.7 to 6.7 during the late 1800's. However, the variance associated with the estimation of inferred pH was greater than the observed shift. Lake T, a clear water alkaline lake, had maintained its pH between 7.7 and 8.2 over the last 80 years.

DICKMAN, M., J. FORTESCUE, M. OUELLET, J. TERASMAE, and I. THOMSON. 1981. Estimating the rate of lake acidification using pH indicator diatome. Can. Tech. Rep. Fish. Aquat. Sci.

Le pH déduit de la présence de diatomés et celui observé dans les lacs ont été comparés pour 27 différents lacs canadiens au nord du lac Supérieur. Le pH déduit des diatomées planctoniques varie d'une demi-unité de pH de celui observé dans 13 des 27 lacs (48%) et d'une marge d'une unité de pH sur 20 (74%) des lacs étudiés. En faisant l'examen d'échantillons de sédiments provenant de 3 des 27 lacs étudiés, on a déduit le pH des lac à des profondeurs successives (i.e. en fonction du temps) en se basant (comme ci-haut) sur la composition des espèces de diatomés ainsi que sur leur abondance relative. La vitesse de changement du pH dans le lac a été estimée en

comparant le pH déduit de la population de diatomés dans les régions de faible et de grande profondeur des échantillons de sédiments. Les horizons du césium 137 et du pollen d'*Ambrosia* sont utilisés pour estimer la vitesse de déposition dans les lacs ainsi qui l'âge des échantillons de sédiments. L'utilisation des études autécologiques de Husted sur les diatomés comme indicateurs de pH permet de conclure que le lac B, un lac riche en humus au nord du lac Supérieur, n'a pas subi d'acidification rapide au cours des quarante dernières années. Le lac F, presque neutre, a subi une diminution de pH de 8.7 a 6.7 vers la fin du 19e siècle. On remarque cependant que la variance associée à l'estimation du pH déduit est plus importante que les fluctuations observées. Le lac T, possédant une eau claire mais alcaline, a maintenu son pH entre 7.7 et 8.2 au cours des dernières années.

TOXICITY OF THE INSECTICIDE PERMETHRIN TO NYMPHS OF THE BURROWING MAYFLY *HEXAGENIA RIGIDA* (EPHEMEROPTERA: EPHEMERIDAE)

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FRIESEN, M.K., T.D. GALLOWAY, and J.F. FLANNAGAN. 1981. Toxicity of the insecticide permethrin to nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera: Ephemeridae). Can. Tech. Rep. Fish. Aquat. Sci.

Water and sediment contained in 1-L glass beakers, in which *Hexagenia rigida* nymphs were being cultured, or to which nymphs would be added, were treated with permethrin at levels which may occur at aerial field application rates of 0.6 and 7.3 g/ha. Mortality of nymphs was monitored at 1, 4 and 10 wks post-treatment. Initial permethrin concentrations in water were estimated by gas liquid chromatography to be 0.6 and 7.6 µg/L (0.6 and 7.6 ppb) at the respective "field" application rates. After 24 h and 7 days levels in sediment were determined by measuring ^{14}C permethrin to be approximately 30-50 µg/kg (30-50 ppb) dry weight at the higher treatment level. Exposure of nymphs to contaminated conditions for a 24-h period immediately following treatment resulted in a final mortality at the respective treatment levels of 27.8 ± 16.94% and 88.0 ± 16.18%. When nymphs were exposed indefinitely to contaminated conditions final mortality was 74.2 ± 10.93% and 100.0% at the respective treatment levels. Treatment related mortality was not significantly different ($P = 0.05$) at the various check times, and much of the mortality could be attributed to lethal effects of permethrin in water. Exposure of nymphs to sediment which had been contaminated at the above levels 8 days previously resulted in 23.3 ± 15.28% and 44.4 ± 8.39% mortality at the one week check time and 45.0 ± 4.05% and 100.0% mortality at the ten week check time at the respective treatment levels. This mortality was apparently due to exposure of nymphs to permethrin associated with the sediment.

FRIESEN, M.K., T.D. GALLOWAY, and J.F. FLANNAGAN. 1981. Toxicity of the insecticide permethrin to nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera: Ephemeridae). Can. Tech. Rep. Fish. Aquat. Sci.

Des béchers de verre de 1 ℓ contenant de l'eau et des sédiments dans lesquels étaient cultivées les nymphes *Hexagenia rigida* ou seraient ajoutées ces mêmes nymphes ont été traités avec de la perméthrine à des niveaux qui peuvent se recontrer dans des applications aériennes aux taux de 0.6 et 7.3 g/ha. On a enregistré la mortalité des nymphes une, quatre et dix semaines après le traitement. Les concentrations initiales de perméthrine dans l'eau ont été mesurées par chromatographie liquide de gaz et estimées à 0.6 et 7.6 µg/ℓ (0.6 et 7.6 ppb) dans les conditions décrites plus haut. Après 24 h et

7 jours, le niveau de perméthrine a été déterminé en mesurant le ^{14}C-perméthrine. On trouve une valeur approximative de 30-50 µg/kg (30-50 ppb) poids sec pour le traitement de 7.3 g/ha. Une exposition des nymphes à des conditions contaminantes pour une période de 24 h immédiatement après le traitement résulte en un taux de mortalité de 27.8 ± 16.94% et 88.0 ± 16.18%. Une exposition indéfinie aux conditions contaminantes résulte en une mortalité finale de 74.2 ± 10.93% et 100% aux taux de traitement respectifs. Le taux de mortalité relié au traitement n'est pas significativement différent ($P = 0.05$) pour les différents temps d'observation. La cause majeure de mortalité peut être attribuée aux effets létaux de la perméthrine dans l'eau. Une exposition des nymphes à des sédiments qui ont été contaminés aux taux mentionnés ci-haut 8 jours avant le traitement résulte en un taux de mortalité de 23.3 ± 15.28% et 44.4 ± 8.39% après une semaine et 45.0 ± 4.05% et 100% après 10 semaines. Cette mortalité est apparemment due à l'exposition des nymphes à la perméthrine associée aux sédiments.

ADENYLATE ENERGY CHARGE AND ATPASE ACTIVITY OF LOBSTER (*HOMARUS AMERICANUS*) DURING SUBLETHAL EXPOSURE TO ZN^{++}

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HAYA, K., B.A. WAIWOOD, and D.W. JOHNSTON. 1981. Adenylate energy charge and ATPase activity of lobster (*Homarus americanus*) during sublethal exposure to Zn^{++}. Can. Tech. Rep. Fish. Aquat. Sci.

Two biochemical parameters which are potentially useful as indicators of sublethal effects of xenobiotics are the adenylate energy charge [AEC = (ATP + 0.5 ADP) ÷ (ATP + ADP + AMP)] and adenosine triphosphatase (ATPase) activity. AEC is a measure of the metabolic energy state of the animal and is a prime factor in controlling catabolic and anabolic processes. Sodium, potassium dependent ATPase (Na, K ATPase) is generally considered to be the prime mediator of ion transport across cell membranes.

Lobsters (*Homarus americanus*) were exposed to a sublethal concentration of Zn^{++} in a flow-through system at $10^{\circ}C$ for 96 h, then transferred to normal sea water for 168 h. The concentration of Zn^{++} in the exposure tanks was 60 ppm at the beginning of the experiment, declined to 25 ppm by 12 h, and stayed constant for the remainder of the exposure phase.

Only 2 deaths occurred (at 48 h and 96 h) out of 60 lobsters during the exposure phase. No deaths occurred during depuration or with the controls. Tail muscle, gill and hepatopancreas samples were taken periodically and freeze-clamped in liquid nitrogen and analyzed for Zn^{++}, AEC and ATPase activity. Total dissection time was less than two min.

The AEC (± standard error of the mean) of the tail muscle, hepatopancreas and gill from 0-hour control lobsters was 0.920 (± 0.17), 0.860 (± 0.24), and 0.877 (± 0.066), respectively. The total concentrations of adenine nucleotides were of the order muscle>>hepatopancreas>gills. No significant differences (t-test) from control values of AEC were found in any of the tissues sampled up to the end of the 96 hours of exposure.

The Na, K ATPase activity was inhibited by Zn^{++}, dropping from 4.51 µmole P_i/mg protein/h for control lobsters to 1.50 µmole P_i/mg protein/h after 96 hours of exposure to Zn^{++}. The decrease in Na, K ATPase became significant (t-test, $P<0.1$) after 12 h of exposure to Zn^{++}. Residual ATPase activity decreased from 2.25 µmole P_i/mg protein/h for control lobsters to 1.01 µmole P_i/mg protein/h. The decrease in activity became significant (t-test, $P<0.01$) after 72 h of exposure to zinc. Both Na, K ATPase activity and residual ATPase activity failed to recover to control levels after 168 h in normal sea water.

The concentration of Zn^{++} in the gills increased from 126 ppm (µg/g dry wt) for controls to 670 ppm after 6 h and 2570 ppm after 96 h of exposure to Zn, then fell to 675 ppm after 168 h of depuration. This indicated that the failure of the ATPases to recover was probably due to inhibition by the residual Zn in the gills rather than insufficient biosynthesis of new enzyme.

HAYA, K., B.A. WAIWOOD, and D.W. JOHNSTON. 1981. Adenylate energy charge and ATPase activity of lobster (*Homarus americanus*) during sublethal exposure to Zn^{++}. Can. Tech. Rep. Fish. Aquat. Sci.

L'énergie de charge de l'adénylate [AEC = (ATP + 0.5 ADP) ÷ (ATP + ADP + AMP)] et l'activité de la triphosphatase d'adénosine (ATPase) sont deux paramètres biochimiques potentiellement utiles comme indicateurs des effets sublétaux de facteurs xénobiotiques. L'AEC est une mesure de l'état d'énergie métabolique des animaux et un facteur primaire de contrôle des processus catabolique et anabolique. L'ATPase dépendant du sodium et du potassium (Na, K ATPase) est généralement considérée comme étant la médiatrice primaire du transport ionique à travers les membranes celulaires.

Des homards (*Homarus americanus*) furent exposés à des concentrations sublétales de Zn^{++} dans un système à circulation d'eau (non-statique) à 10°C pour une période de 96 jours, puis transférés dans de l'eau de mer normale pendant 168 heures. La concentration de Zn^{++} dans les réservoirs expérimentaux était de 60 ppm au début, diminua à 25 ppm après 12 heures et demeura constante pour la période d'exposition restante.

Deux homards sur soixante sont morts durant la période d'exposition (à 48 h et à 96 h). Aucune mortalité n'est survenue pendant l'épuration ou dans les contrôles. Des échantillons du muscle de la queue, de branchies et d'hépatopancréas ont été extraits périodiquement, congelés dans de l'azote liquide et analysés pour déterminer la quantité de Zn^{++} et l'activité de l'AEC et de l'ATPase. Le temps total de dissection a été enregistré comme étant moins de 2 minutes.

Les valeurs d'AEC (± erreur standard sur la moyenne) du muscle de la queue, de l'hépatopancréas et des branchies des homards au temps initial de contrôle (0-heure) sont de 0.920 (± 0.17), 0.860 (± 0.24) et 0.877 (± 0.066), respectivement. Les concentrations totales des nucléotides d'adénine sont les suivantes: muscle>>hépatopancréas>branchies. Aucune différence significative (*t*-test) n'a été trouvée entre les valeurs de contrôle de l'AEC et les tissus échantillonnés, et ce jusqu'à la fin de la période d'exposition de 96 heures.

L'activité du Na, K ATPase est inhibée par Zn^{++}, passant de 4.51 µmole P_i/mg protéine/h à 1.50 µmole P_i/mg protéine/h chez les homards de contrôle, après 96 heures d'exposition à Zn^{++}. La diminution du Na, K ATPase devient significative (*t*-test, $P<0.1$) après 12 h d'exposition au Zn^{++}. L'activité résiduelle de l'ATPase diminue de 2.25 µmole P_i/mg protéine/h à 1.01 µmole P_i/mg protéine/h chez les homards de contrôle. La diminution de l'activité devient significative (*t*-test, $P<0.01$) après 72 heures d'exposition au zinc. L'activité de Na, K ATPase ainsi que l'activité résiduelle de l'ATPase n'augmentent pas aux niveaux des contrôles après 168 h dans l'eau de mer normale.

La concentration de Zn^{++} dans les branchies augmente de 126 ppm (µg/g poids sec) à 670 ppm après 6 h, 2570 ppm après 96 h d'exposition au Zn et diminue à 675 ppm apres 168 h d'épuration. Ceci indique que l'échec des ATPases à remonter aux niveaux initiaux est probablement dû à l'inhibition du Zn résiduel dans les branchies plutôt qu'à une biosynthèse insuffisante de nouveaux enzymes.

BIOCONCENTRATION OF VANADIUM IN AMERICAN FLAGFISH OVER ONE REPRODUCTIVE CYCLE

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HOLDWAY, D.A., J.B. SPRAGUE, and J.G. DICK. 1981. Bioconcentration of vanadium in American flagfish over one reproductive cycle. Can. Tech. Rep. Fish. Aquat. Sci.

Previous work by the authors has established the sublethal no-effect level for American flagfish to be about 0.08 mg/L, or roughly 0.007 of the lethal level for this species. One other aspect of vanadium toxicity needed to be covered in order to assess danger to fish, and that was its potential for bioaccumulation.

Groups of 30 one-week-old larvae were started in continuous exposures to various constant levels of vanadium. The fish were sampled after 28 days and 96 days, by which time they had matured and spawned for about 25 days. Eggs were reared at the same constant levels of vanadium and the resultant 30-day-old larvae of the second generation were also assessed for vanadium content. Analysis of vanadium was carried out on a Perkin Elner Model 503 atomic absorption spectrophotometer with a model HGA 2100 graphite furnace.

Flagfish accumulated 9.6-30.6 μg/g of vanadium (whole body dry weight) during chronic exposures to 0.17, 0.48, and 1.50 mg/L of the metal. These were significantly higher than residues of about 0.46 and 4.01 μg/g for control fish and those exposed to 0.041 mg/L; those two residue levels were not significantly different. The bioconcentration range of 10-121 times was similar to metals such as zinc, and much lower than methylmercuric chloride and most organic toxicants. Residue levels were directly related to exposure concentrations but bioconcentration factors were inversely related. Both residues and bioconcentration factors were directly related to exposure time up to 70 days after which they remained stable. Danger from bioaccumulation of vanadium in fish was judged to be very low.

HOLDWAY, D.A., J.B. SPRAGUE, and J.G. DICK. 1981. Bioconcentration of vanadium in American flagfish over one reproductive cycle. Can. Tech. Rep. Fish. Aquat. Sci.

Des ouvrages antéreurs par les auteurs ont établi le niveau sublétal de non-effet pour le Joranella floridae comme étant environ 0.08 mg/ℓ ou approximativement 0.007 du niveau létal pour cette espèce. Un autre aspect de la toxicité du vanadium doit être étudié de façon à établir le danger menaçant le poisson: le potentiel de bioaccumulation.

Des groupes de 30 larves, âgées de 1 semaine, ont subi des expositions continues à différentes concentrations constantes de vanadium. Les poissons ont été échantillonnés après 28 et 96 jours, temps pendant lequel ils avaient atteint leur maturité et s'étaient reproduits pendant 25 jours. Les oeufs ont été laissés au même niveau constant de vanadium et les larves de 30 jours de la deuxième génération ont aussi été analysées pour leur contenu en vanadium. L'analyse du vanadium a été executée sur un spectrophotomètre d'absorption atomique Perkin Elner Model 503 avec une fournaise au graphite modèle HGA 2100.

Une accumulation de 9.6-30.6 µg/g de vanadium dans le Jordanella floridae (poids sec calculé pour le corps entier) est mesurée dans le cas d'expositions chroniques de 0.17, 0.48 et 1.50 mg/ℓ au métal. Ces derniers sont significativement plus élevés que les résidus d'environ 0.46 et 4.01 µg/g pour les poissons de contrôle ainsi que ceux exposés à une concentration de 0.041 mg/ℓ; ces deux niveaux de résidus ne sont pas significativement différents. L'échelle de bioconcentration de 10-121 fois est similaire à d'autres métaux tels que zinc et beaucoup plus basse que le chlorure de méthyle mercurique et la plupart des substances toxiques organiques. Les niveaux de résidus sont directement reliés aux concentrations d'exposition alors que les facteurs de bioconcentration sont reliés de facon inverse. Les facteurs de résidus et de bioconcentration sont directement reliés au temps d'exposition jusqu'à 70 jours, puis ils demeurent stables. Le danger issu de la bioaccumulation du vanadium dans le poisson est considéré comme étant très faible.

CHRONIC TOXICITY OF A MIXTURE OF 7 METALS TO FLAGFISH IN SOFT, ACID WATER

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Field workers have documented recruitment failures in the fish populations of acid lakes. They have questioned whether the elevated heavy metal concentrations which accompany decreased pH are a factor in the disappearance of fish from those lakes. Although the effects of low pH on fish are well documented and the toxicities of some heavy metals have been studied, little is known of the chronic toxicity of heavy metals in soft, acid water. Field observations and on-site bioassays have not separated the effects of low pH and increased heavy metals from those of low pH alone.

Laboratory testing of the American flagfish (*Jordanella floridae*) over 1.3 generations in soft (5.0 ppm as $CaCO_3$), acid (pH 5.8) water gives clear evidence that the increased metals content of acid lakes could be a major cause of the observed reproductive failures. Fish were exposed to acid water containing a mixture of Al, Mn, Zn, Fe, Ni, Cu and Pb at 0.05, 0.16 or 0.5 of the levels of those metals that would be usual in a lake at pH 5.8. Test results were compared to acid (pH 5.8) and neutral (pH 7.3) controls.

Reproductive failure was complete at pH 5.8 with 0.5 of the metal-levels. Fry died within six days of exposure, adults transferred into the treatment did not spawn and hatchability of transferred eggs was reduced. Pre-exposure of eggs and fry to sublethal concentrations increased fry resistance time from two to six days in lethal conditions.

At pH 5.8 with 0.16 of the usual metal levels there was reduced size of surviving juveniles and adult females of the first generation, delayed spawning, and decreased survival and size of second-generation juveniles. At pH 5.8 and 0.05 metal-levels there was delayed spawning and a transient reduction in size of first-generation females. No significant effects were seen in the pH 5.8 controls.

Other studies have shown that the threshold of reproductive impairment in fish is between pH 5.5 and pH 6.0. This experiment shows that for waters in this pH range, metals in association with low pH may be the primary cause of reproductive falure, and not low pH alone.

This study is part of a series planned to evaluate specific field observations of toxicity, on the basis of laboratory bioassays. Further studies will determine the contribution of individual metals to the toxicity of the mixture, the role of naturally occurring chelating agents in reducing heavy metal toxicity and the effect of lake neutralization by liming on heavy metal toxicity.

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Des recherchistes travaillant sur le terrain ont documenté les échecs de recrutement dans les populations de poissons des lacs acides. Ils se sont demandés si les concentrations en métaux lourds élevées qui accompagnent une diminution du pH contribuent à la disparition des poissons dans ces lacs. Malgré l'abondance de documentation concernant les effets d'un pH faible sur les poissons et les études menées sur la toxicité de certains métaux lourds, la toxicité chronique de ces derniers dans les eaux douces et acides nous est à peu près inconnue. Les observations sur le terrain ainsi que les essais biologiques in situ n'ont pas séparé les effets d'un faible pH et d'une augmentation des métaux lourds de ceux d'un faible pH par lui-même.

Des test de laboratoire sur l'espèce Jordanella floridae pendant 1.3 génération, en eau douce (5.0 ppm de $CaCO_3$) et acide (pH 5.8) montre clairement qu'une augmentation de la quantité de métaux dans les lacs acides pourrait être une cause majeure des échecs au niveau de la reproduction. Les poissons ont été exposés à de l'eau acide contenant un mélange de Al, Mn, Zn, Fe, Ni, Cu et Pb à 0.05, 0.16, ou 0.5 des taux de métaux habituellement présent dans le lac au pH 5.8. Les résultats ont été comparés à des contrôles acides (pH 5.8) et neutres (pH 7.3)

A un pH de 5.8 et un niveau de 0.5 en métal on note l'échec total de la reproduction. Les alevins sont morts à l'intérieur d'une période de 6 jours après l'exposition, les adultes transférés dans le même traitement ne démontrent pas la moindre tentative de frai et l'éclosion des oeufs transférés est réduite. Une préexposition des oeufs et des alevins à des concentrations inférieures à la dose létale augmente le temps de résistance des alevins de deux à six jours dans des conditions létales.

A un pH de 5.8 et à 0.16 des niveaux de métaux habituellement présents la grosseur des juvéniles survivant et des femelles adultes de la première génération diminue, la reproduction est retardée et de nombre des survivants et la grosseur des juvéniles de la seconde génération diminuent. A un pH de 5.8 et un niveau de métaux de 0.05, la reproduction est retardée et la grosseur de la première génération de femelles diminue temporairement. On ne constate pas d'effets significatifs dans les contrôles de pH 5.8.

D'autres études montrent que le seuil de détérioration des fonctions reproductives du poisson se situe entre pH 5.5 et 6.0. Cette expérience montre que pour les eaux dont le pH se situe entre ces limites, les méteaux, en association avec un pH peu élevé peuvent être la cause primaire des échecs reproduction plutôt que le pH peu élevé par lui-même.

Cette étude fait partie intégrante d'une série d'articles planifiée pour évaluer les observations spécifiques sur la toxicité, à partir d'essais biologiques en laboratoire. Des études ultérieures pourront déterminer la contribution individuelle de différents métaux sur la toxicité de mélanges, le rôle des agents chélateurs dans la réduction de la toxicité des métaux lourds ainsi que l'effet de neutralisation du lac par l'addition de $CaCO_3$ sur la toxicité des métaux lourds.

THE EFFECT OF pH ON THE DISTRIBUTION OF CRUSTACEAN ZOOPLANKTON IN 158 QUEBEC LAKES

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JOUBERT, G. and L. TOUSIGNANT. 1981. The effect of pH on the distribution of crustacean zooplankton in 158 Quebec lakes. Can. Tech. Rep. Fish. Aquat. Sci.

A preliminary study of the zooplankton in 158 lakes located across Quebec south of the 52nd parallel was conducted during the summer of 1980. Once the cladocerans and the copepods had been inventoried and classified, the communities were analyzed from the point of view of their response to the phenomenon of lake acidification. A total of 48 species were found — 29 species of cladocerans and 19 species of copepods. Species were classified by frequency of occurrence and by the number of lakes in which they were dominant. The largest number of species occurred at pH values 6.1-8.0, with breaks in their distribution appearing at pH values lower than 5.5 and higher than 8.0. At a pH of less than 6.0, the frequency of occurrence drops at a faster rate, although down to a pH of 5.0 the number and composition of the species remain essentially the same. Below this point only the most tolerant species remain. The same phenomenon occurs at a pH of more than 8.0. At a pH of less than 5.0 the communities consist of only 3 to 5 species; many species are no longer present, and the tolerant species occur at a frequency of only 3.4 percent, becoming progressively rarer.

JOUBERT, G. and L. TOUSIGNANT. 1981. The effect of pH on the distribution of crustacean zooplankton in 158 Quebec lakes. Can. Tech. Rep. Fish. Aquat. Sci.

Durant l'été 1980, une étude préliminaire a été menée sur le zooplancton de 158 lacs situés à travers le Québec, au sud du 52° parallèle. La réaction à l'acidification des lacs a été analysée chez les différentes communautés. Les espèces de cladocères et copépodes présentes ont été répertoiriées. Un total de 48 espèces furent dénombrées, 29 de cladocères et 19 de copépodes. Ces espèces furent classifiées selon leur fréquence d'occurrence et la nombre de lacs où elles étaient dominantes. Le plus grand nombre d'espèces se rencontrent du pH 6.1 à 8.0, avec des discontinuités dans leurs distributions aux valeurs de pH plus basses que 5.5 et plus élevées que 8.0. A un pH plus bas que 6.0, la fréquence d'occurrence diminue rapidement mais en se rapprochant du pH 5.0, le nombre et la composition des espèces présentes demeurent essentiellement les mêmes. A un pH plus bas, on trouve seulement les espèces les plus tolérantes. Le même phénomène se produit à un pH plus élevé que 8.0. A un pH plus bas que 5.0, les communautés ne contiennent que 3 ou 5 espèces et les espèces tolérantes ont une fréquence de seulement 3.4%, devenant de plus en plus rares.

METALLOTHIONEIN MEASUREMENT BY POLAROGRAPHY AS AN INDICATOR OF HEAVY METAL EXPOSURE IN SALMONIDS

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OLAFSON, R.W., M. ROCH, A.J. MCCARTER, and A.T. MATHESON. 1981. Metallothionein measurement by polarography as an indicator of heavy metal exposure in salmonids. Can. Tech. Rep. Fish. Aquat. Sci.

Metallothionein contents in livers of rainbow trout obtained at various points downstream from a copper and zinc mine on Vancouver Island were measured by differential pulse polarography.

The tissue concentration was related to the extent of heavy metal contamination, the quantity of heavy metal in the low molecular weight fraction of gel filtered liver cytosol and to the amount of copper and cadmium bound to high molecular weight proteins.

The data indicate that the polarographic measurement of metallothionein is a rapid and sensitive technique in assessing biochemical response to heavy metals. Work is underway to relate metallothionein levels and synthetic rates to lethal tolerance with the intention of relating intracellular heavy metal burden and survival capacity by this single determinant.

OLAFSON, R.W., M. ROCH, A.J. MCCARTER, and A.T. MATHESON. 1981. Metallothionein measurement by polarography as an indicator of heavy metal exposure in salmonids. Can. Tech. Rep. Fish. Aquat. Sci.

La métallothionéine contenue dans le foie de truites arc-en-ciel obtenues en aval de différentes locations situées dans des régions minières (cuivre, zinc) sur l'île de Vancouver, a été mesurée par polarographie à pulsation différentielle.

La concentration dans les tissus a été comparée à l'étendue de la contamination par les métaux lourds, à la quantité de métaux lourds dans la fraction de poids moléculaire bas de la suspension de cellules du foie filtrée par gel et à la quantité de cuivre et de cadmium liés aux protéines à poids moléculaire élevé.

Les résultats obtenus indiquent que la mesure polarographique de métallothionéine est une technique rapide et précise pour l'évaluation des réactions biochimiques aux métaux lourds. Des travaux sont en cours afin d'établir la relation entre les niveaux de métallothionéine, les taux synthétiques et la tolérance létale dans l'intention de relier le fardeau intracellulaire de métaux lourds et la capacité de survie par ce seul facteur.

UPTAKE AND PERSISTENCE OF PERMETHRIN BY FISH, VEGETATION, AND HYDROSOIL

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RAWN, G.P., D.C.G. MUIR, and G.R.B. WEBSTER. 1981. Uptake and persistence of permethrin by fish, vegetation and hydrosoil. Can. Tech. Rep. Fish. Aquat. Sci.

In 1979 and 1980, outdoor artificial ponds were treated with ^{14}C permethrin at 0.028 kg/ha (15 μg/L). Uptake of permethrin by fathead minnows, *Pimephales promelas*, duckweed, and hydrosoil was monitored by TLC-autoradiography, HPLC, and liquid scintillation counting. Rapid loss of permethrin from the water coincided with the detection of five degradation products in the water at concentrations never exceeding 2.0 μg/L. The products were *cis*- and *trans*-cyclopropyl acid, phenoxybenzoic acid, and phenoxybenzyl alcohol, and an unknown non-cleaved product of permethrin. Permethrin was readily sorbed by duckweed, fathead minnows, and hydrosoil. In 1979 and 1980 the maximum permethrin concentrations reached in the duckweed were 30 and 55 μg/g, respectively, which decreased to less than 0.1 μg/g by 29 days post-treatment. A growth inhibition of the duckweed was observed in the ponds treated with permethrin. Permethrin concentration in the fish reached 1.1 mg/kg but was undetectable by four weeks post-treatment. No fish mortality was observed. In 1979, permethrin residues in the hydrosoil reached a maximum of 40 μg/kg and in 1980, 120 μg/kg. Permethrin was persistent in the hydrosoil with 0.25 μg/kg detected at 420 days (1979) and 4.9 μg/kg at 323 days (1980). *Cis*-permethrin was more persistent in the hydrosoil than the *trans*-permethrin. The results indicated that permethrin in water was short-lived at an application rate of 15 μg/L as a result of the rapid degradation of permethrin in the water and sorption of permethrin by the hydrosoil, vegetation, and fish. However, at over one year post-treatment, permethrin residues were still detected in the hydrosoil.

RAWN, G.P., D.C.G. MUIR, and G.R.B. WEBSTER. 1981. Uptake and persistence of permethrin by fish, vegetation and hydrosoil. Can. Tech. Rep. Fish. Aquat. Sci.

En 1979 et 1980, des bassins articifiels extérieurs ont été traités avec de la perméthrine - C^{14} à un taux de 0.028 kg/ha (15 μg/ℓ). L'absorption de perméthrine par les menés à grosse tête, *Pimephales promelas*, les lentilles d'eau et l'hydro-terroir a été mesurée par chromatographie en couche mince (auto-radiographie/TLC), HPLC (chromatographie liquide à haute pression) et par comptage à scintillation liquide.

Des pertes considérables de perméthrine dans l'eau coincident avec la détection de cinq produits de dégradation, lesquels n'excèdent jamais une concentration de 2.0 μg/ℓ. Les produits ci-haut mentionnés sont identifiés comme étant l'acide *cis*- et *trans*-cyclopropylique, l'acide phenoxybenzoique, l'alcool phénoxybenzylique ainsi qu'un produit non-fractionné et inconnu de la perméthrine. Les lentilles d'eau, les menés à grosse tête ainsi que l'hydrosol absorbent facilement la perméthrine. En 1979 et 1980, les concentrations maximales de perméthrine atteintes dans les lentilles d'eau étaient de 30 et 55 μg/g, respectivement, et ont diminué à moins de 0.1 μg/g après 29 jours de traitement. Une inhibition de croissance des lentilles d'eau est observée dans les bassins traités avec de la perméthrine. La concentration de perméthrine dans les poissons atteint 1.1 mg/kg mais devient impossible à détecter après 4 semaines de traitement. Aucun poisson n'est mort. En 1979, les résidus de perméthrine dans l'hydrosol atteignaient une concentration de 40 μg/kg. Et en 1980, ils étaient de 120 μg/kg. La perméthrine dans l'hydrosol persistait à une concentration de 0.25 μg/kg après 420 jours (expérience 1979) et de 4.9 μg/kg après 323 jours (expérience 1980). La *cis*-perméthrine est plus persistante que la *trans*- du même nom dans l'hydrosol. Les résultats obtenus indiquent que la perméthrine a une courte durée de vie dans l'eau lorsqu'appliquée au taux de 15 μg/ℓ et ceci résulte de la dégradation rapide de la perméthrine dans l'eau et de la sorption de la perméthrine par l'hydrosol, la végétation et les poissons. Il est à spécifier, cependant, qu'après une année de traitement, des résidus de perméthrine étaient encore détectables dans l'hydrosol.

EFFECTS OF WATER HARDNESS, INORGANIC MERCURY AND ZINC ON UPTAKE OF WATERBORNE METHYLMERCURY IN RAINBOW TROUT (*SALMO GAIRDNERI*)

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RODGERS, D.W. and F.W.H. BEAMISH. 1981. Effects of water hardness, inorganic mercury and zinc uptake of waterborne methylmercury in rainbow trout (*Salmo gairdneri*). Can. Tech. Rep. Fish. Aquat. Sci.

We obtained simultaneous measurements of oxygen consumption and uptake of waterborne methylmercury of rainbow trout (*Salmo gairdneri*) swimming at sustained speeds at 10°C. The relationships between oxygen consumption and uptake of ^{203}Hg-labeled methylmercury were examined in hard water (385 ± 5 mg/L total hardness) with and without addition of either inorganic mercury or zinc and in soft water (30 ± 3 mg/L total hardness) with and without the addition of inorganic mercury.

Oxygen consumption was not affected at the concentrations of labeled methylmercuric chloride (< 5 µg/L), mercuric chloride (≈ 30 µg/L) or zinc sulphate (≈ 300 µg/L) employed. The efficiency of uptake of methylmercury relative to oxygen, E(p/o), was estimated as: $E(p/o) = (\Delta P \div [P])/(Q \div [O_2])$ where ΔP is the rate of uptake of methylmercury by the fish (ng CH_3Hg/h), $[P]$ is the mean concentration of methylmercury (ng CH_3Hg/mL), Q is the rate of oxygen consumption of the fish (mg O_2/h) and $[O_2]$ is the mean oxygen concentration (mg O_2/mL).

The efficiency of uptake of methylmercury relative to oxygen was significantly ($P < 0.05$) affected by water hardness, and the addition of either of inorganic mercury or zinc. The E(p/o) obtained in hard water without the addition of any inorganic metals was 0.28 ± 0.2 (mean ± SE, n = 11), a ratio not significantly different from the value of approximately 0.25 previously obtained for trout at 10 and 20°C in hard water. In soft water, without the addition of inorganic metals, the E(p/o) increased approximately three-fold to 0.82 ± 0.2 (n = 9). When mercuric chloride was added with the labeled methylmercury, the E(p/o) increased to approximately 1.6 in both hard and soft water. The E(p/o) measured in hard water with added inorganic mercury (1.57 ± 0.15, n = 11) was not significantly different ($P < 0.05$) from that obtained in soft water with added inorganic mercury (1.64 ± 0.15, n = 12). In hard water, the E(p/o) decreased slightly to 0.15 ± 0.2 (n = 4) when zinc was added with the labeled methylmercury.

The reduction in efficiency of uptake of methylmercury observed in hard water may in part explain the commonly observed reduction in toxicity of metals in hard water. As the principle ions responsible for water hardness, Ca^{++} and Mg^{++}, also markedly affect cellular permeability, these changes in uptake likely reflect alterations at the cellular level. The increased efficiency of uptake of methylmercury observed in soft water suggests that the reported increased methylmercury concentrations attained by fish in lakes of low alkalinity and pH may be due to the effects of hardness rather than pH.

As inorganic mercury may be expected to be in considerable excess of methylmercury in natural waters, the observed facilitation of methylmercury uptake by inorganic mercury is of considerable environmental significance. The marked facilitation of methylmercury uptake by mercuric chloride but not zinc sulphate indicates that this facilitation is not a general effect of divalent metals but may be specific to the mercuric ion species complex. A saturation of gill uptake may be indicated by the similar efficiencies of methylmercury uptake obtained in hard and soft water with added mercuric chloride. As mercuric chloride has been observed to bind to the gill membrane it is probable that the observed changes in uptake reflect alterations at the cellular level. The concentrations of mercuric chloride used have been reported to increase mucus release in rainbow trout and this increased mucus may serve as a methylmercury trap. The reduction in efficiency of methylmercury uptake with added zinc may indicate a competitive inhibition and is consistent with reports that another divalent metal, copper, was antagonistic to the toxicity of methylmercury to fish.

ROGERS, D.W. and F.W.H. BEAMISH. 1981. Effects of water hardness, inorganic mercury and zinc uptake of waterborne methylmercury in rainbow trout (*Salmo gairdneri*). Can. Tech. Rep. Fish. Aquat. Sci.

Des mesures simultanées de consommation d'oxygène et d'absorption de méthyle de mercure en milieu aquatique par la truite arc-en-ciel (*Salmo gairdneri*) nageant à vitesse soutenue à 10°C ont été obtenues. La relation entre la consommation en oxygène et l'absorption de ^{203}Hg sous forme de méthyle de mercure a été établie à partir d'examens en eau dure (385 ± 5 mg/ℓ dureté totale), avec ou sans addition de mercure inorganique ou de zinc et en eau douce (30 ± 3 mg/ℓ dureté totale) avec ou sans addition de mercure inorganique.

La consommation d'oxygène n'est pas influencée par les concentrations de chlorure de méthyle de mercure (< 5 µg/ℓ), de chlorure de mercure ($\simeq 30$ µg/ℓ) ou de sulfate de zinc ($\simeq 300$ µg/ℓ) employées. Le taux d'efficacité de l'absorption du méthyle de mercure relativement à l'oxygène, E(p/o), a été estimé comme étant $E(p/o) = (\Delta P \div [P])/(Q \div [O_2])$ ou ΔP est le taux d'absorption de méthyle de mercure par le poisson (ng CH_3Hg/h), $[P]$ est la concentration moyenne de méthyle de mercure (ng CH_3Hg/mℓ), Q est le taux de consommation d'oxygène du poisson (mg O_2/h) et $[O_2]$ est la concentration moyenne d'oxygène (mg O_2 / mℓ).

L'efficacité de l'absorption du méthyle de mercure, relativement à l'oxygène, est influencée significativement par la dureté de l'eau ($P < 0.05$) et l'addition de mercure inorganique ou de zinc. Le E(p/o) obtenu en eau dure, sans l'addition de métaux inorganiques, est 0.28 ± 0.2 (moyenne ± SE, n = 11) , un rapport non-significativement différent de la valeur de 0.25 obtenue précédemment pour la truite à 10°C et 20°C, en eau dure. En eau douce, sans l'addition de métaux inorganiques, le E(p/o) a subi une augmentation approximative de trois fois sa valeur, portant celle-ci à 0.82 ± 0.2 (n = 9) . Une addition de mercure sous forme de méthyle de mercure augmente la valeur de E(p/o) de 1.6 dans l'eau dure et l'eau douce. Le E(p/o) mesuré en eau dure additionnée de mercure inorganique (1.57 ± 0.15, n = 11) n'est pas différent de façon significative ($P < 0.05$) de celui obtenu en eau douce avec une même addition de mercure (1.64 ± 0.15, n = 12). En eau dure, le E(p/o) diminue légèrement jusq'à une valeur de 0.15 ± 0.2 (n = 4) avec addition combinée de zinc et de méthyle de mercure.

Une réduction de l'efficacité de l'absorption de méthyle de mercure en eau dure peut, en partie, expliquer la diminution communément observée de la toxicité des métaux en eau dure. Comme les principaux responsables de la dureté de l'eau, les ions Ca^{++} et Mg^{++}, affectent aussi la perméabilité de la cellule de façon drastique, ces changements au niveau de l'absorption reflètent probablement des changements au niveau cellulaire. L'augmentation de l'efficacité d'absorption du méthyle de mercure observée en eau douce suggère que les concentration élevées de méthyle de mercure chez les poissons de lacs à faibles alcalinité et pH sont dues aux effets de la dureté de l'eau plutôt qu'au pH.

Comme on doit s'attendre à ce que la quantité de mercure inorganique dépasse de beaucoup la quantité de méthyle de mercure dans les eaux naturelles, la facilitation de l'absorption du méthyle de mercure par le mercure inorganique telle qu'observée, revête une importance environnementale considérable. La facilitation de l'absorption du méthyle de mercure par le chlorure mercurique n'est pas le reflet d'un effet général associé aux ions métalliques bivalents puisqu'on ne constate pas de facilitation d'absorption en présence de sulfate de zinc; mais elle peut être spécifique aux complexes de mercure sous différentes formes ioniques. Des efficacités similaires d'absorption de méthyle de mercure dans l'eau dure et douce, en présence de chlorure de mercure, peuvent indiquer une saturation de l'absorption des branchies. Le chlorure de mercure ayant déjà été observé attaché à la membrane branchiale, il est probable que les changements observés dans l'absorption soient le reflet de changements au niveau cellulaire. On a rapporté que les concentrations de méthyle de mercure utilisées augmentent la libération de mucus chez la truite arc-en-ciel et cet excès de mucus peut servir à piéger celui-ci. La diminution dans l'efficacité d'absorber le méthyle de mercure en présence de zinc, peut indiquer une inhibition compétitive. Cette possibilité est consistante avec les rapports que le cuivre, un autre métal divalent, est antagoniste à la toxicité du méthyle de mercure chez le poisson.

THE SENSITIVITY OF SIX STRAINS OF UNICELLULAR ALGAE *SELENASTRUM CAPRICORNUTUM* TO SIX REFERENCE TOXICANTS

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SCHOENERT, R., P. COUTURE, C. THELLEN, and R. VAN COILLIE. 1981. The sensitivity of six strains of unicellular algae *Selenastrum capricornutum* to six reference toxicants. Can. Tech. Rep. Fish. Aquat. Sci.

Investigators have been active in considering other trophic levels than fish as indicators of water quality and several suggested the use of algae and promoted the development of algal bioassay procedures. The PAAP test has been extensively used as a measurement of eutrophication and to evaluate the potential fertility of a natural water. The species of algae used in many cases has been *Selenastrum capricornutum*. We are active in defining new norms for the use of this species in an algal bioassay to measure the response of the environment to a wastewater. The use of reference toxicants as indicators of variability in sensitivity between stocks of test organisms is documented for fish and macroinvertebrate bioassays. This investigation was undertaken as part of a study to evaluate the sensitivity of 6 strains of *Selenastrum capricornutum* to 6 chemical compounds which have been accepted as reference toxicants in aquatic bioassays.

The *Selenastrum* species obtained were from stock cultures being maintained at the Freshwater Institute in Winnipeg, Man., Canada Centre for Inland Waters in Burlington, Ont., the University of Quebec at Montreal, P.Q., Environment Canada in Longueuil, P.Q., the Environmental Protection Service in Quebec City and the National Institute for Scientific Research in Quebec City. The reference toxicants tested were sodium azide, cadmium nitrate, phenol, sodium lauryl sulfate, potassium dichromate and cupric sulfate.

The test procedure followed was as suggested by the U.S. EPA with certain modifications. Test volumes were reduced to 40 mL and replicates were done as triplicates. The flasks were initially inoculated with 1×10^3 cells/mL and incubated for 14 days at 5400 lux with a 16 h light 8 h dark photoperiod at $24^{\circ}C \pm 2^{\circ}C$. On day 15, algal numbers were determined in all flasks using a Coulter Counter.

Fifty percent inhibition of growth (CI50) was determined for each of the six strains of *Selenastrum* to the six reference toxicants. The methods of binomial estimation, moving average and probit analysis were applied to obtain these values. The six strains of *Selenastrum* tested demonstrated a wide range of response to the six reference toxicants. Mean CI50 values for a specific reference toxicant displayed coefficients of variation as high as 75% (i.e. $CdNO_3$) when the six strains were compared. Further, estimates of CI50 values varied depending on the statistical analysis applied. The work suggests that

in order to adopt a standard procedure for the use of this test organism to monitor toxicity in the environment, more attention must be given to the strain of *Selenastrum* utilized and the method adopted to calculate the CI50 value.

SCHOENERT, R., P. COUTURE, C. THELLEN, and R. VAN COILLIE. 1981. The sensitivity of six strains of unicellular algae *Selenastrum capricornutum* to six reference toxicants. Can. Tech. Rep. Fish. Aquat. Sci.

Des chercheurs ont entrepris des études considérant des niveaux trophiques autres que ceux des poissons comme indicateurs de la qualité de l'eau. Plusieurs d'entre eux ont suggéré l'utilisation d'algues et ont mis en mouvement le développement de processus d'essais biologiques des algues. Le test PAAP est employé très souvent pour mesurer le niveau d'eutrophisation et pour évaluer le potentiel de fertilité de l'eau à l'état naturel. L'espèce d'algue utilisée dans la majeure partie des cas est *Selenastrum capricornutum*. Beaucoup d'efforts sont entrepris pour définir de nouvelles normes d'utilisation de cette espèce dans les essais biologiques d'algues, pour mesurer la réponse de l'environnement aux eaux usées. L'utilisation de substances toxiques de référence comme indicateurs de variation dans la sensibilité des différents groupes d'organismes servant au test est documentée pour les poissons et les macroinvertébrés. Cette recherche fait partie intégrante d'une étude d'évaluation de la sensibilité de 6 de races de *Selenastrum capricornutum* à 6 différents composés chimiques jugés acceptables comme substances toxiques de référence pour ces essais biologiques aquatiques.

Les cultures de *Selenastrum* ont été obtenues de l'Institut d'Eau Douce, Winnipeg, Manitoba, du Centre Canadien des Eaux Intérieures à Burlington, Ontario, de l'Université du Québec à Montréal, Québec, d'Environnement Canada, à Longueuil, Québec, du Service de Protection de l'Environnement, à Québec, Québec et de l'Institut National de Recherche Scientifique, à Québec, Québec. Les substances toxiques de référence testées sont l'azide de sodium, le nitrate de cadmium, le phénol, le sulfate lauryle de sodium, le bichromate de potassium et le sulfate cuivrique.

Le protocole utilisé est celui suggéré par E.P.A. (E.U.) avec certaines modifications. Les volumes-test ont été réduits à 40 mℓ et testés en triplicata. Les flacons ont été inoculés (initialement) avec 1×10^3 cellules/mℓ et incubés pendant 14 jours à 5,400 lux, $24^{\circ}C \pm 2^{\circ}C$, avec une photopériode de 16 heures de clarté et 8 de noirceur. Au quinzième jour, le nombre d'algues contenu dans les ballons a été calculé à l'aide d'un compteur Coulter.

On a déterminé l'inhibition de croissance 50% (CI50) pour chacune des 6 races de *Selenastrum* impliquées dans l'étude pour les 6 substances toxiques de référence. Les méthodes d'estimation binomiales, de déplacement moyen et d'analyses probit ont été appliquées pour obtenir ces valeurs. Les six races de *Selenastrum* testées démontrent beaucoup de variations dans leurs réactions aux six substances toxiques de référence. Les valeurs moyennes de CI 50 pour une des références montrent des coefficients de variation allant jusqu'à 75% (i.e. $CdNO_3$) quand les six races étaient comparées entre elles.

De plus, les estimés des valeurs de CI50 varient selon la méthode statistique employée. Ce travail suggère que dans le but d'adopter une méthode standard pour l'utilisation de ces tests de contrôle de toxicité de l'environnement, plus d'attention doit être portée à la race de *Selenastrum* utilisée ainsi qu'à la méthode employée pour calculer la valeur de CI50.

BIOASSY MONITORING OF STEEL PLANT TREATED PROCESS WATER AT STELCO INC.

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SCHULDT, A.A. and R.P. SCROGGINS. 1981. Bioassay monitoring of steel treated process water at Stelco Inc. Can. Tech. Rep. Fish. Aquat. Sci.

A comprehensive environmental monitoring and impact study is described for evaluating treated process water from Stelco's Lake Erie Works (L.E.W.). The steel making facility located near the village of Nanticoke on the north shore of Lake Erie commenced operations in 1980 using state-of-the-art water treatment technology.

An on-site mobile laboratory was used for conducting a series of lethal and sublethal bioassay tests to study the effects of treated process water on a variety of standard aquatic test organisms and important native species. Test species include: rainbow trout *Salmo gairdneri*, American flagfish *Jordanella floridae*, green algae *Chlorella vulgaris*, water flea *Daphnia magna*, yellow perch *Perca flavescens*, small-mouth bass *Micropterus dolomieui* and amphipod *Gammarus fasciatus*.

The paper describes test methods and experimental results from experiments conducted using treated water which discharges to a nearshore water regime.

SCHULDT, A.A. and R.P. SCROGGINS. 1981. Bioassay monitoring of steel treated process water at Stelco Inc. Can. Tech. Rep. Fish. Aquat. Sci.

Une étude détaillée de l'impact sur d'environnement est décrite pour évaluer les eaux usées traitées de Lake Erie Works (L.E.W.) de Stelco. Cette usine de fabrication d'acier est située près du village de Nanticoke, sur la rive nord du lac Erié. Elle est entrée en opération en 1980 et est munie des plus récents développements technologiques pour le traitement des eaux usées.

Une série de bioassais létaux et sub-létaux a été effectuée sur place, dans un laboratoire mobile, pour étudier l'effet des eaux usées traitées sur une variété d'organismes - tests standard ainsi que sur des espèces indigènes importantes. Les espèces testées comprennent: la truite arc-en-ciel *Salmo gairdneri*, le *Jordanella floridae*, l'algue verte *Chlorella vulgaris*, la puce d'eau *Daphnia magna*, la perchaude *Perca flavescens*, l'achigan à petite bouche *Micropterus dolomieui* et l'amphipode *Gammarus fasciatus*.

Cet article décrit les méthodes employées et les résultats expérimentaux obtenus en testant les eaux usées traitées qui se déversent près de la berge.

THE EFFECTS OF ATRAZINE IN A LAKE ECOSYSTEM USING LARGE VOLUME (125 M^3) *IN SITU* ENCLOSURES

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DAY (SMITH) K., N.K. KAUSHIK, K.R. SOLOMON, and C.T. CORKE. 1981. The effects of atrazine in a lake ecosystem using large volume (125 m^3) *in situ* enclosures. Can. Tech. Rep. Fish Aquat. Sci.

Triplicate, *in situ* enclosures or limnocorrals (5 x 5 x 5 m deep) were treated with the herbicide atrazine at 0.2 mg/L or 2.0 mg/L during the summer of 1981. Three others, controls, were treated with only solvent. The experiment was part of a larger study to determine methodology for evaluating the effects of pesticides on lake communities.

Significant decreases in levels of dissolved oxygen due to reduced algal populations occurred throughout the water column in all atrazine-treated corrals. Large phytoplankton species (mainly diatoms, *Dinabryon*) and filamentous green algae on the inner walls of the plastic curtains were reduced in corrals treated with both concentrations. Nutrient levels (total dissolved phosphorus, total dissolved organic nitrogen, dissolved organic and inorganic carbon) remained the same in corrals treated with 0.2 mg/L of atrazine but increased in corrals treated with 2.0 mg/L of atrazine. The additional availability of nutrients may have allowed one or more unidentified, unicellular green algal species to increase their populations for a period of time following treatment with the herbicide. Further investigations are necessary to determine what factors are involved in this increase.

Atrazine did not significantly affect populations (number/L) of crustaceans and rotifers at either concentration. Indirect removal of some algal species by the pesticide may have contributed to a reduced species diversity of zooplankton by elimination of a food source for some of the filter-feeding crustaceans.

DAY (SMITH), K., N.K. KAUSHIK, K.R. SOLOMON, and C.T. CORKE. 1981. The effects of atrazine in a lake ecosystem using large volume (125 m^3) *in situ* enclosures. Can. Tech. Rep. Fish. Aquat. Sci.

Durant l'été 1981, des enclos *in situ* et en trois exemplaires, en milieu lacustre, ont été traités avec de l'herbicide atrazine à 0.2 mg/ℓ ou 2.0 mg/ℓ. Trois autres enclos, les contrôles, ont été traités seulement avec du solvant.

Cette expérience en fait partie d'une plus grande qui a pour but de développer une méthodologie pour évaluer les effets des pesticides sur les communautés lacustres. La réduction des populations d'algues produit des diminutions significatives de l'oxygène dissous dans la colonne d'eau des enclos traités à l'atrazine. Les espèces de macrophytoplancton (spécialement les diatomées, *Dinabryon*) et les algues vertes filamenteuses, que l'on trouve sur la face intérieure des rideaux de plastique, sont réduites dans les enclos traités aux deux concentrations d'atrazine. Les niveaux d'éléments nutritifs (total de phosphore dissous, total d'azote organique dissous, carbone organique et inorganique dissous) demeurent les mêmes dans les enclos traités avec 0.2 mg/ℓ d'atrazine mais augmentent dans ceux traités avec 2.0 mg/ℓ d'atrazine.

La disponibilité additionnelle des éléments nutritifs a pu permettre à une ou plusieurs espèces non-identifiées d'algues unicellulaires d'augmenter leurs populations pour une certaine période de temps, après le traitement. De plus amples recherches sont nécessaires pour déterminer les facteurs impliqués dans cette augmentation.

L'atrazine n'a pas augmenté significativement (nombre/ℓ) les populations de crustacés et de rotifères à aucune des concentrations. L'élimination indirecte de quelques espèces d'algues par le pesticide peut avoir contribué à la réduction de la diversité des espèces de zooplancton en enlevant une source de nourriture.

POSSIBILITY OF SHORT TERM RESISTANCE TO HEAVY METALS BY AQUATIC ORGANISMS

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VAN COILLIE, R., C. THELLEN, and J.C. DOL. 1981. Possibility of short term resistance to heavy metals by aquatic organisms. Can. Tech. Rep. Fish. Aquat. Sci.

In the course of various bioassays, the following histochemical mechanisms were elucidated:

i) when lobsters *Homarus americanus* are exposed to lead for 48 h, this heavy metal partially replaces calcium and magnesium in the external cuticle, either directly or via the gills;
ii) in macrophytes *Scirpus americanus* exposed to mercury and lead for 72 h, these two heavy metals precipitate into submicroscopic granules in the vessels of the rhizome and the stalk and do not affect the chlorophyll cells;
iii) in fish *Carassius auratus* in contact with cadmium for 12 h, it was observed that this element is temporarily and partially stored in the scales.

These data support the hypothesis of a "mineral sink" inside a "mineral turnover" within aquatic organisms. These organisms can use this system, on a short term basis and in a limited manner, to resist the toxic effects of heavy metals.

VAN COILLIE, R., C. THELLEN, and J.C. DOL. 1981. Possibility of short term resistance to heavy metals by aquatic organisms. Can. Tech. Rep. Fish. Aquat. Sci.

Dans le cours de différents essais biologiques, les mécanismes histochimiques suivants furent élucidés:

i) quand le homard *Homarus americanus* est exposé au plomb pour une période de 48 h, ce métal lourd remplace partiellement le calcium et le magnésium dans la cuticule externe, d'une façon directe ou en passant par les branchies;

ii) quand le macrophyte *Scirpus americanus* est exposé au mercure et au plomb pendant 72 h, ces deux métaux lourds précipitent sous forme de granules sous-microscopiques à l'intérieur des ballons contenant le rhizome et la tige mais n'affectent pas les cellules chlorophylliennes;

iii) quand le poisson *Carassius auratus* est en contact avec le cadmium pendant 12 h, on observe une accumulation partielle et temporaire de cadmium dans les écailles.

Ces données supportent l'hypothèse de l'existence d'un bassin accumulateur de minéraux dans un cycle minéral à l'intérieur des organismes aquatiques. Ces organismes peuvent utiliser ce système, à court terme et de façon limitée, pour pouvoir résister aux effets toxiques des métaux lourds.

UNIFORM METHODS FOR EXPOSURE REGIMES IN AQUATIC TOXICITY EXPERIMENTS WITH CHEMICALLY DISPERSED PETROLEUM OILS

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WELLS, P.G., J.W. ANDERSON, and D. MACKAY. 1981. Uniform methods for exposure regimes in aquatic toxicity experiments with chemically dispersed petroleum oils. Can. Tech. Rep. Fish. Aquat. Sci.

Many different approaches have been used in marine laboratories for preparing and analysing test solutions and dispersions of hydrocarbons and dispersants, and oiled sediment mixtures for toxicological assessments. This has made much of the published work difficult to compare and summarize, and has often led investigators to incorrect conclusions regarding the joint toxicity of mixtures of hydrocarbons and dispersants.

A working document is being prepared, presenting recommended approaches and principles for water and sediment studies with these materials. Specific items are also discussed, such as dispersant:oil ratios, reagent mixing, choice of oils and dispersants, degree of "weathering" of the oils, and analysis of hydrocarbons.

This document is meant to stimulate the adoption of more uniform procedures for preparing test solutions and other preparations with oils and dispersants. It is particularly meant to be useful for researchers starting new work with these materials. We feel that this document is urgently needed because of previously ignored pleas internationally on this topic, and because of the many new toxicity studies presently being initiated in various countries. It is intended to be complementary to the American Society for Testing and Materials (ASTM) "Proposed Standard Practice for Determining the Acute Toxicity of Chemical Dispersants and Chemically Dispersed Oil to Marine Organisms", in preparation through the ASTM F20.13.02 Task Group.

WELLS, P.G., J.W. ANDERSON, and D. MACKAY. 1981. Uniform methods for exposure regimes in aquatic toxicity experiments with chemically dispersed petroleum oils. Can. Tech. Rep. Fish. Aquat. Sci.

Plusieurs approches différentes ont été utilisées dans la préparation et l'analyse de solutions-test, de dispersions d'hydrocarbones et de dispersants, de mélanges huileux de sédiments, pour les estimations toxicologiques dans les laboratoires orientés dans les études marines. Cette diversité

rend les ouvrages publiés difficiles à comparer et à analyser. Ceci explique également la fréquence des conclusion incorrectes obtenues par les chercheurs en ce qui a trait à la toxicité de mélanges d'hydrocarbones et de dispersants.

Un document de travail qui recommande des approches et principes dans les études de l'eau et des sédiments est actuellement sous préparation. Différents éléments spécifiques y sont discutés: le rapport dispersant:huile le mélange des réactifs, le choix d'huiles et de dispersants, le degré de désagrégation des huiles et l'analyse des hydrocarbones.

Ce document veut stimuler l'adoption de procédures plus uniformes dans les préparation de solutions test ainsi que celles des huiles et dispersants. Il est particulièrement utile pour les chercheurs qui commencent du nouveau travail avec ces matériaux. On voit l'urgence liée au besoin d'un tel outil de travail pour deux raisons principales: les plaidoyers internationaux, auparavent ignorés, sur ce sujet et l'abondance de nouvelles études de toxicité en cours dans différents pays du monde. On propose ce document comme complément à l'ASTM (The American Society for Testing and Materials): "Méthodes standards de détermination de la toxicité des dispersants chimiques et des huiles chimiquement dispersées sur les organismes marins", actuellement en préparation sous la tutelle du groupe de travail de l'ASTM F20.13.02.

COMPARISON OF ALGAL BIOASSAY TECHNIQUES IN TOXICITY STUDIES

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WONG, P.T.S., Y.K. CHAU, and D. PATEL. 1981. Comparison of algal bioassay techniques in toxicity studies. Can. Tech. Rep. Fish. Aquat. Sci.

Five techniques were used to study the effects of a metal mixture on freshwater green alga, *Ankistrodesmus falcatus*. The first three techniques involved batch culture and the remaining two were continuous culture. The first one was the conventional ^{14}C-$NaHCO_3$ technique in which the cells were exposed to the metal mixture for various periods of time and the effect of the toxicant was determined by the amount of ^{14}C-$NaHCO_3$ taken up by the cells after a 4 h incubation. The second technique involved exposing the cells to the toxicant in a flask. At various time intervals, a small volume of the medium was withdrawn and the number of cells was counted in the microscope. In the third technique, a side-arm flask was used to grow the algae. The amount of algal growth in the presence and the absence of metal mixture was conveniently monitored by inserting the side-arm flask into a Klett-Summerson colorimeter and recording the optical density. The two continuous culturing techniques involved a turbidostat and a chemostat. In the turbidostat, the metal mixture was exposed to a constant and pre-determined cell number in an automated algal cultivation controller (ACC-5, Techtum Instrument). The cell concentration in the turbidostat was controlled by a photocell. When the cell number exceeds the pre-determined value, the solenoid valve of the photocell would open and release a fresh medium from the reservoir to the culture vessel to dilute the cells. The chemostat involved a constant supply of medium and the toxicant to a growth chamber. The effect of the toxicant on the growth kinetics of the alga was determined by cell counts.

The results from these five techniques all indicated the toxic effects of the metal mixture on the green alga. The advantages and disadvantages of these techniques in algal toxicity studies will be discussed.

WONG, P.T.S., Y.K. CHAU, and D. PATEL. 1981. Comparison of algal bioassay techniques in toxicity studies. Can. Tech. Rep. Fish. Aquat. Sci.

Cinq techniques ont été utilisées pour étudier les effets d'un ensemble de métaux sur les algues vertes d'eau fraiche *Ankistrodesumus falcatus*. Les trois premières méthodes consistaient en groupes de cultures, alors que les deux autres étaient des cultures continues. La première technique utilisée était celle de ^{14}C-$NaHCO_3$ dans laquelle les cellules furent exposées à un mélange de métaux pendant des périodes de temps variables. On a déterminé l'effet de toxicité en mesurant la quantité de ^{14}C-$NaHCO_3$ absorbée par les cellules après 4 heures d'incubation. Dans la seconde technique, les cellules

étaient exposées au milieu intoxicant dans un ballon. A différents intervalles de temps, on retirait un faible volume de solution et on comptait le nombre de cellules. Dans la troisième technique, un ballon à double embouchure fut utilisé pour la croissance des algues. La croissance des algues fut évaluée en insérant l'embouchure de côté du ballon dans un Colorimètre Klett-Summerson et en mesurant la densité optique. Les deux techniques de culture continue comprenaient un turbidostat ainsi qu'un chimiostat. Dans le turbidostat, le mélange de métaux fut exposé à un nombre constant et prédéterminé de cellules dans un contrôleur automatique de culture des algues (ACC-5, Techtum Instrument). La concentration de cellules dans le turbidostat était contrôlée par une cellule-photo. Quand le nombre de cellules excède la valeur prédéterminée, la valve solénoïde de la cellule-photo s'ouvre pour laisser s'échapper un volume de solution fraîche du réservoir au milieu de culture, de façon à diluer le milieu cellulaire. Le chimiostat assure un approvisionnement constant de solution et de substance toxique à la chambre de croissance. L'effet du composé toxique sur la cinétique de croissance des algues a été déterminé par comptes de cellules.

Les résultats de ces cinq techniques démontrent les effets toxiques du mélange de métaux sur les algues vertes. Les avantages et désavantages de ces techniques dans les études de toxicité sur les algues sont discutés.

METAL LEVELS IN AN AQUATIC ECOSYSTEM

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WREN, C.D. and H.R. MACCRIMMON. 1981. Metal levels in an aquatic ecosystem. Can. Tech. Rep. Fish. Aquat. Sci.

Acid precipitation has resulted in increased metal levels in the waters of many Precambrian Shield lakes. Given the toxicity of many of these elements, and the widespread nature of the problem, it is essential to identify those metals which bioaccumulate through the food chain. Samples representing a total aquatic ecosystem were collected from a Precambrian Shield lake which is not subject to cultural disturbances except possibly atmospheric input. The samples include: sediments, clams, several fish species, beaver, racoon, and otter. Each sample has been analyzed for over twenty naturally occurring elements, including: Hg, Zn, Mg, Mn, Al, and Cd. The concentration of these elements will be discussed relative to accumulation in the food chain.

WREN, C.D. and H.R. MACCRIMMON. 1981. Metal levels in an aquatic ecosystem. Can. Tech. Rep. Fish. Aquat. Sci.

Les précipitations acides ont donné lieu à une augmentation des niveaux de métaux dans les eaux de plusieurs lacs du bouclier précambrien. Prenant en considération l'effet toxique de beaucoup de ces éléments ainsi que la gravité toujours grandissante de ce problème, il devient essentiel d'identifier les métaux qui s'accumulent au cours de leur transmission dans la chaîne alimentaire. Des échantillons représentatifs d'un écosystème aquatique complet ont été extraits d'un lac du bouclier précambrien qui n'est pas sujet aux perturbations causées par le processus de mise en culture, à l'exception possible des apports atmosphériques. Les échantillons comprennent: des sédiments, des pétoncles, différentes espèces de poissons, des castors, des ratons laveurs et des loutres. Chaque échantillon a été analysé dans le but de mesurer 20 éléments naturels du milieu, entre autres: Hg, Zn, Mg, Mn, Al et Cd. La concentration de ces éléments sera discutée relativement à leur accumulation dans la chaîne alimentaire.

REPORT OF THE QUESTIONNAIRE WORKSHOP ON "TRAINING OF AQUATIC TOXICOLOGISTS"

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INTRODUCTION

A questionnaire requesting information on academic background, current work responsibilities and ideas to improve training was circulated at the workshop. Of the 150 questionnaires given out, 41 were returned and this report presents an analysis of the data supplied. Where possible, the data have been reduced to tabular form and are presented in this manner for ease of interpretation.

RESULTS

Responses to question 1, area of formal training, are given in table 1.

Table 1: Area of formal training.

Area	Number Responding	% Responding
General Biology	18	44
Aquatic Biology	7	17
Marine/Fisheries Biology	3	7
Physiology	3	7
Microbiology	2	5
Environmental Physiology	1	2
Pharmacology	1	2
Environmental Biology	1	2
Chemistry	1	2
Engineering	1	2
Agriculture	1	2
Psychology	1	2
Zoology	1	2

In response to questions 2 and 3, area of current work and area of work responsibility the data in table 2 were obtained.

Table 2: Area of work and area of responsibility.

Area of formal training	Area of work and number	Number in each area of responsibility				
		Technical, data collection	Supervision, data analysis	Enforcement, regulation	Academic teaching	Policy formation
Microbiology	Biology 2	1	-	-	1	-
Physiology	Biology 2	1	-	1	-	-
Marine/Fish Biology	Biology 3	1	1	1	-	-
Aquatic Biology	Biology 6	1	4	-	-	1
	Toxicology 1	-	-	-	-	1
Biology	Biology 17	10	3	2	1	1
	Toxicology 1	-	-	-	-	1

Question 4 requested data on the employer and, of the total of 41 persons responding;

59% work for government
24% for universities
10% for consultants and
7% for industry.

Questions 5 to 28 requested information on areas in which formal training was lacking. The answers were graded from 1 = (strongly disagree that training was lacking) to 5 (strongly agree that training was lacking). This information was analysed by calculation of the arithmetic mean response. The standard deviation was used as a measure of diversity of response. In view of the small sample size, all the work and work training areas could not be meaningfully analysed, and only those working in biology and trained either in biology or aquatic biology were analysed as distinct groups. The results are presented in table 3.

The mean response of the whole group for all questions was 3.00 and, on the basis that this score is indicative of a satisfactory educational exposure to the area, the following were identified as needing more attention

Table 3: An analysis of areas in which formal training was lacking.

	Mean response (standard deviation)		
Area of training	Whole group N = 41	Biologists in Biology (N = 7)	Aquatic Biologists in Biology (N = 17)
Physics	2.9 (1.4)	2.5 (1.5)	2.9 (1.2)
Biology (general)	1.8 (1.1)	1.8 (1.1)	1.7 (0.8)
Biology (aquatic)	2.5 (1.4)	2.9 (1.5)	1.4 (0.8)
Biochemistry	2.7 (1.1)	2.5 (0.9)	3.0 (1.3)
Limnology	2.9 (1.4)	3.3 (1.5)	1.9 (0.9)
Hydrology	3.8 (1.3)	3.8 (1.3)	3.0 (1.3)
Soil Science	3.7 (1.3)	4.3 (0.8)	3.6 (1.4)
Animal Physiology	2.6 (1.3)	2.8 (1.0)	2.0 (1.0)
Plant Physiology	3.1 (1.2)	2.8 (1.0)	2.7 (1.1)
Toxicology	3.7 (1.3)	4.2 (1.1)	3.4 (1.1)
Inorganic Chemistry	2.8 (1.1)	3.0 (1.0)	2.6 (0.8)
Organic Chemistry	2.7 (1.1)	3.2 (1.1)	2.1 (0.9)
Physical Chemistry	3.8 (1.1)	3.2 (1.3)	2.6 (1.0)
Analytical Chemistry	3.3 (1.3)	3.7 (1.1)	3.4 (1.0)
Pharmacology	4.2 (0.9)	4.5 (0.8)	4.0 (1.0)
Statistics	2.9 (1.3)	3.2 (1.2)	1.7 (0.8)
Mathematics	2.3 (1.1)	2.6 (1.1)	2.4 (1.0)
Computer Science	3.9 (1.3)	3.8 (1.1)	2.3 (1.4)
Economics	3.6 (1.4)	3.4 (1.3)	3.1 (1.3)
Resource Management	3.8 (1.3)	4.0 (1.1)	3.1 (1.7)
Political Science	4.1 (1.2)	3.8 (1.3)	3.1 (0.8)
Ecology	2.1 (1.1)	2.2 (1.1)	1.6 (0.8)

Mean response 3.00 all areas

The following were also listed as receiving too little attention;

Law (2x)
Engineering (2x)
Ethics
Philosophy
Epidemiology
Pathology
Entomology

during the training of aquatic toxicologists; Pharmacology, Political Science, Computer Science, Resource Management, Physical Chemistry, Toxicology, Hydrology and Economics. The major areas identified as already receiving too much attention were; General Biology, Mathematics and Ecology.

The responses to questions 29 to 32 are analysed in table 4 using a similar method to that used in table 3.

Table 4: General areas of importance to aquatic toxicology.

Area	Mean Response	Standard Deviation
Statistics	1.9	0.8
Ecology	1.8	0.9
Physiology	2.0	1.2
Chemistry	1.9	1.1

The rating system ranged from 1 (most important) to 5 (least important). The fact that all areas received almost equal ratings suggested their equal importance to toxicology and further supported the data in table 3 which indicated that areas such as chemistry should receive more attention and areas such as ecology less attention. In other words the perceived surfeit in ecology training is not merely due to a lack of importance of ecology in the work area, but to a genuine superfluity.

Of the responses to question 33 "Given time and support, would you be interested in obtaining a course work (no thesis) Masters in Toxicology?", 50% (N = 40) said yes.

CONCLUSIONS

In any survey of this type it is very difficult to determine if the response is indeed typical or representative of the whole group or "population". In this case ca. 30% of those attending the Workshop responded to the questionnaire, however this only represents 10% of the Canadian section of the mailing list for the Workshop. In addition, the location of the workshop does have an influence on the type of work and the educational background of the people attending. It is obvious that the workshop was composed predominantly of persons from Ontario and that a higher proportion than normal were probably employed as students or in research/teaching in the University system.

Taking into account the possible bias inherent in this survey, it is possible to generate the following conclusions,

1) The majority of those working in aquatic toxicology were educated in general or aquatic biology.
2) The majority of those working in aquatic toxicology do work that is biologically orientated and mainly involves data collection and data analysis.
3) The majority of those working in aquatic toxicology work for the Government and Universities
4) Educational resources for those working in aquatic toxicology are perceived to be lacking in the areas of Pharmacology, Political Science/Law, Computer Science, Resource Management, Physical Chemistry, Toxicology, Hydrology and Economics. A surfiet of educational resources is perceived to exist in the areas of General Biology, Mathematics and Ecology.
5) There is a perceived need for postgraduate training (via a non-thesis masters program) in the area of Toxicology.

NOTES ON THE WORKSHOP - 96 H LC50 - WHAT NEXT?

Dmitry Stone

Biological Services, MacLaren, Engineers Planners & Scientists Inc.,
1220 Sheppard Ave. E., Suite 100,
Willowdale, Ontario, M2K 2T8

INTRODUCTION

Gordon Craig gave a quick history of the test and outline of its characteristics: a practical screening tool, non species-specific, provided an integrative approach but avoided detailed chemistry, increasing regulatory use required standardization and protocols were developed on the basis of experience, the 100% LC50 was identified as an ititial goal and it is estimated that half of industries can meet this now. He then posed the question for the workshop, "What next?" and suggested updated goals; protection of the eco-system, and particularly man-protection of water quality for all desired uses and improvement where appropriate and *methods*; use of biological monitors to protect biological uses - investigative monitoring programs.

He said that EPS is still seeking a simple comprehensive screening test while continuing to use the 96 h LC50 as a regulatory tool.

He suggested that the current need is for a simple reference test which will protect the most sensitive use of receiving waters. It must be practical, quick, economical and standardized.

Following G. Craig's opening remarks, comments were invited from the audience. These are reported as recorded:

R. Watts (B.C.)

On the west coast the LC50 is the major test. The EPS is still wading through effluents. There is not much point using other tests until they know what is the "soup" of effluent.

As far as government is concerned the LC50 for trout will continue to be used.

Using 25% of the 96 h LC50 for guppy - courtship was affected, but there was no effect on the temperature preference. Liked the behavioural tests and get an idea for long-term loading.

Behavioural changes were related to tissue loads, so may be a good method would be to measure bioaccumulation.

Should look more often at primary productivity - testing is too weighted towards animals.

LC50 is a good test for industries with small environmental laboratories.

D. Stone

LC50 for oil fractions, useful monitoring but need ideas to define the tests.

R. Scroggins

LC50 - good - but design the tests to fit the situation.

G. Craig

Asked what type of monitoring program Beak would design for Stelco in light of experience. Indefinite answer: would repeat, delete and propose other tests, i.e., wouldn't do the same battery of tests again.

Brian Hammond (Alberta)

Ames test shouldn't be used solely on groups. Use a tiered system to include Microtox; Ames - cell culture - whole animal. Start with microtox - if chemical fails this, go on to the next test, e.g. Daphnia.

M. Taylor

The U.S. and OECD have been talking about toxicity testing for years. What good will our discussions do in this workshop - how is it proposed that our thoughts reach the appropriate ears?

A test which is recognized across the country is needed. Care is needed as problems from 30 years ago are still present.

Must decide the level of protection to be obtained. As we progress we become more aware of problems, e.g., Hg. Have to look at waters which have a natural high level of a chemical.

Norman Bermingham

The trout assay has handicapped us in developing more meaningful tests - it is a "sacred cow". Must be sure that any new standards test which may be developed do not become more "sacred cows". Must make sure that the right questions are asked, e.g., when considering bioaccumulation - must not standardize before the test is ready.

P. Hodson

Have to prove that chemicals are toxic before they can be controlled or banned.

There are differences in the quality of the water between lab and field tests. The lab is not the field. Have to end up with population studies - could become another "sacred cow".

We should be looking at "pure" research in more depth as the basic information is needed in applied studied.

The public is becoming quite sophisticated and they are understanding more. We are asked - "is the chemical safe" - but it is very difficult to give an answer.

Many tests have been proposed based on risk models, i.e., not straight toxicity. Look at such things as birth defects - mutation rates, cancer.

D. Stone

Are we suggesting a complete reorientation of our thinking?

G. Craig

P. Hodson's idea (population studies) is difficult to interpret. We need to have narrow reference testing included in ecosystem assessments.

Progress is needed in the state-of-the-art.

Should tie in biological response with probability -risk assessment.

(Hodson?) (speaker not identified in notes)

In Canada at the present time, no one has set down exact definitions on how safe we want to be. All government departments and university agencies are off on their own interests. It should be stated what tests and why they are being done. Also, we must decide how much we are willing to pay. At the moment, there is a very disorganized approach.

Maybe Canada can cope with our own problems, but we are not isolated from the rest of the world.

Pollution legislation is old, vague and open to interpretation.

It the government set a total environmental degradation limit of 10%; the industries already there and new ones coming in would all have to improve their treatment - or new industries would have to go elsewhere.

In Quebec, there is a difference from federal requirements. It has to be proved that something is susceptible to damage. A wide range of tests can be used. Algae, microtox are accepted in court - need a wide variety of tests. Ames test has been shown to give a high percentage of incorrect responses.

What level of chemical in water is related to a level in fish which is safe for eating - what is the risk level - and for how many years down the road?

G. Craig

The public will ask for all the protection that they can get, i.e., zero risk.

Within the biological framework, we are working at the 5% level which is intolerable to the public.

In car driving (i.e., the public has a choice) evaluation is different. There is no control by the public over exposure to environmental pollutants.

We need to get more sophisticated in our tests; evaluations. Need one very sensitive test to compare with medium and low sensitivity, so that when we get enough data in the years ahead, we can collate them all and thus end up with fewer tests about which we know a great deal.

D. Stone

What level would you be looking at for a very sensitive test? chromosome, cell, whole organism?

G. Craig

The answer depends on what is thought to be sensitive. Have to work hard to get a good data base using lots of tests.

There is a feeling that there are restrictions in the tests we use. Industry - can never use one test - have to evaluate the seriousness of the problem. Determine the types of risks, determine which test to use.

D. Stone

Government agencies are very rigid at times and quote, for example, a single level of a metal without taking the volume of the receiving water into account - or the whole ecosystem effect, etc.

Need a battery of tests designed and decide what parts are best for particular problems, e.g., for a new industry.

Workshop is a good place to start thinking - but then what?

LC 50 is still useful where there are many gross problems still to answer: but must be backed up with others. Must get a relationship between the results of many tests and get this in a package for industry regulators.

SUMMARY - WORKSHOP ON FRESHWATER/SALTWATER TOXICITY

P.G. Wells[1,2]

[1]Institute for Environmental Studies, University of Toronto
Toronto, Ontario, M5S 1A4

[2]Marine Ecology Laboratory, Bedford Institute of Oceanograph,
Dartmouth, N.S., B2Y 4A2

Panel Members: J.W. Anderson, M. Speyer, and G.F. Westlake

This workshop addressed several questions on the comparative acute and chronic toxicities of persistent xenobiotics in freshwater, estuarine and marine waters, and the applications of the data and priciples for regulatory and monitoring purposes. There was a lively panel discussion and numerous questions from the other participants.

The major questions, some of which were raised in workshops from 1975 to 1980, were: (1) Generally, are there significant differences in the toxicities of substances between freshwater and saltwater? (2) Are we adequately protecting estuarine and saltwater bodies by screening effluents with freshwater bioassay techniques, or should we proceed with site-by-site, situation-by-situation saltwater evaluations and bioassays? (3) Since most pollutants of concern accumulate greatly in sediments, do we need site-specific, sediment and benthic accumulation and release data, due to recognized differences between the freshwater and saltwater environments? (4) Do we need estuarine and marine water quality criteria in Canada, and if so, which data bases should be used for these criteria?

It was generally agreed that there are many differences in the water properties, types and physiologies of biota and consequently responses to toxicants between fresh and saltwater environments. A very large literature now exists on this topic. However, comparisons of large sets of freshwater and saltwater data (lethal toxicity) on single materials such as heavy metals, dispersants and petroleum hydrocarbons often show no differences or small differences, indicating the potential applicability of large sets of acute freshwater data for the gross regulation of materials entering saltwater. This may indicate that, at the present time, the requirement for suites of saltwater, regulatory, end-of-pipe bioassays on effluents and single materials entering saltwater is unnecessary.

For evaluations beyond the pipe, the panel members urged the use of site-specific experiments, bioassays and biomonitoring approaches; Anderson and Speyer were particularly concerned about modifying factors in the various marine environments, and Westlake felt that field-oriented, reproductive assays should be encouraged. The contamination of sediments and the resulting effects on biota in the different estuarine and marine benthic environments dominated the discussion, and is of major concern.

The general opinion expressed throughout the workshop discussions was that, beyond the initial monitoring and control of major pollutants at source using currently available freshwater bioassays, all materials entering marine waters should be evaluated further with sensitive marine assays and on-site experiments and monitoring. The urgent need for marine water and sediment quality criteria in Canada was recognized and recommended. Such criteria should be based on all available data on the materials in question, with an emphasis on marine data. These criteria should be formulated by the responsible government departments as soon as possible.

This workshop on freshwater/saltwater toxicity emphasized the importance of and challenges involved in comparative aquatic toxicology, and introduced topics that will undoubtedly be discussed again and again in future Aquatic Toxicity Workshops in Canada.

ACKNOWLEDGEMENTS

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WORKSHOP ON
CREDIBILITY OF SCIENCE TO THE PUBLIC

O.P. Dwivedi

Opening Remarks by Professor O.P. Dwivedi, Department of Political
Studies, University of Guelph,
Guelph, Ontario, N1G 2W1

This workshop was about the issue for the growing credibility gap between scientists and the public in Canada. It is known that at the decision-making level, but more particularly in the legislative process, relationship of science to the pressing social and economic issues is not well understood. The situation becomes more murky when discussions on long-range societal issues and needs of the future are held. Is it because the average politician and senior government administrator does not know much about politics? If science appears to have become a non-issue, who should be blamed? Certainly not the politicians and others. As stated by J.J. Schepherd (Executive Director of the Science Council of Canada):

> Science is non-issue because scientists have made it so. The golden era of science in the 1960's engendered a sense of security and certitude in scientists about public acceptance of the national mission of science. This sense of security has recently been rudely and deservedly shattered. The role of science is being questioned. (*issues in Canadian Science Policy*, February 1976, p. 1.)

Because of this, and largely because of the loss of societal focus on the importance of science in the national agenda, "science fails to command political attention".

What politicians want are answers from scientists so that they could arrive at a proper decision; so that they can convince their constituents for the courageous stand they had to take against the trendy social issues or against the politically unacceptable economic issues. But the politicians get confused when they are faced with the lack of proof which scientists can offer in areas such as environmental management or in public health - so the gap is growing between politicians and scientists.

What we need is a continuing dialogue between the scientists and the decision-makers. This dialogue would be held publicly, especially before decisions are made so that suitable modifications could be inserted.

It should be noted that scientists represent one of the best brainpowers of the nation. They are interested in the economic and social well-being of the nation; and, perhaps they can easily convince the public and the decision-makers alike if they put their mind to it. Scientists have moral obligation, professional responsibility, and duty to the society to act responsibly and to explain their work to the public; and to take full part in the decision-making process. Otherwise, public acceptance of their mission will not be fully realized. What is needed now is improved communications between the scientists and policy-makers. They, through their professional associations/societies, would prove that science and technology is capable of enhancing the economy of Canada, suggesting alternate strategies for fighting socio-economic problems, and preparing the nation to face the 21st century. This role will make them responsible and accountable. In the words of John K. Galbraith: "In a scientifically exacting world, scientists must assume responsibility for the consquences of science and technology." Scientists do not live in a value-free world; on the contrary, their work affects us all. Consequently, they must accept their responsibility to the society by discarding apathy and disinterest, and becoming involved citizens. This will help them to fill the credibility gap, as presently felt by the scientific community.

These remarks were then supplemented by a talk given by Michael Keating, environment reporter for the *Globe and Mail*. There were about 75 persons present in the workshop.

WORKSHOP ON DATA BASES FOR AQUATIC TOXICOLOGY

W.M.J. Strachan

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Canada Centre for Inland Waters
Burlington, Ontario
L7R 4A6

Data Bases are simply collection of information and their usefulness is related to whether they provide this information in a format and degree useful to the person enquiring of them. Some are automated, accessible by telephone and telecommunication terminals; others are manual. Some are free or purchased by organizations which in their turn provide the information free of charge; others have charges associated with them - either on an hourly-rate basis or a flat fee basis. Many of the data bases allow searching for the stored information by a variety of parameters other than the chemical identifier - i.e. by toxic level, by physical-chemical parameter, by affected organism. The approach which the individual takes to the bases will be governed by their needs.

There are many such data bases. They have been developed by different organizations to serve many different purposes and the stored data is accessible in a variety of different ways determined by the needs of these organizations. Any individual data base may contain only a part of the information which a researcher may be seeking; others may be very complete but are limited by the number of compounds included. It is up to the individual to familiarize himself/herself with the contents of those which seem most appropriate and to evaluate usefulness. These bases are, however, potential time-savers for researchers who are prepared to examine them thoroughly for their potential to satisfy their own particular information needs. This paper was not intended to be comprehensive nor to provide the necessary instructions on how to contact or access the many data bases. Rather, its purpose was to heighten the awareness of scientists, especially aquatic toxicologists, concerning the existence of data bases which may be of value to them and to solicit comment on the nature of a data base which might be of use to them in hazard assissments of toxic chemicals. These comments should apply not only to the coverage of the bases, but also to data quality, extent of "current" data, accessibility and other factors.

The Toxic Substance Committee of the International Joint Commission's (IJC) Water Quality Board (WQB) undertook an examination of approximately 200 such data bases. In their 1980 Report they list these bases along with the nature of the data and an indication of the mode of access. Their 1981 report will focus on a few of them which are particularly relevant to the Great Lakes ecosystem especially to Annex 12 of the Great Lakes Water Quality Agreement (1978). Another document which gives information on how to access a number of data bases, as well as their nature and cost, is the report for US-EPA prepared by Koba Assoc (1978). Both of these reviews are recommended to the aquatic toxicologist who is unfamiliar with the world of bases and wishes to investigate them further.

Hazard is perceived as a property of a substance that is related to both exposure and effects. Better estimates of both of these aspects may be obtained by measuring them in the environment but in general, scientists and regulators will arrive as decisions on whether a substance represents an environmental or human health hazard on the basis of predictions from laboratory data, an expediency made necessary by available information. Without both exposure and effect, there can be no hazard. Observed exposures, by their very nature, are localized in focus although for some airborne contaminants, international exposure may occur. Data bases which provide information on observed Canadian exposure are few. WATDOC provides a general system, accessible by terminal and telephone coupler, and contains information on a number of bases indicated in Table 1. Also included in this table, but not part of WATDOC, is NAQUADAT, a computerized data base containing information on water quality measurements. A U.S. system, STORET, provides similar exposure data, some of which is relevant to Canadian situations. Release related information (production, sites, uses, disposal, commercial quantities) is important to exposure and there are several relevant data bases also listed.

Table 1: Data Bases Relevant to Observed Canadian Exposure

CENV/ WATDOC	Canadian Environment - scientific and technical literature and reports (including those of Environment Canada) relevant to a variety of topics not restricted to toxic contaminants. Baseline information from BEIRS (Baseline Environmental Information Reference System) is included.
DREF/ WATDOC	Data Reference - Canadian data collections from the Environmental Conservation (formerly Management) Service of Environment Canada. Some other data collections from other federal and provincial agencies are included.
ENV/ WATDOC	Environment - similar in purpose to CENV in the French language although the coverage is not as extensive.
NAQUADAT	National Quality Data System - water quality data mainly for traditional but with some toxic chemical parameters. The data is available by river basin at identified sites.
CCUBE	Chemicals in Canadian Commerce - information on commercial quantities, uses and company locations plus a limited amount of data on physico-chemical properties.
EPSCIS	Environmental Protection Service Chemical Information System - an Ontario region data system intended to collect information on physical-chemical and toxicological properties of chemicals as well as their quantities and uses in commerce. Currently largely contains data on the IJC list of chemicals in the Great Lakes.

Laboratory data on characteristics, information on exposure, and effects of toxic chemicals is the one which can most profitably be examined with respect to providing input to Canadian hazard assessments. Such data are considered universal to a chemical and are applicable wherever the chemical may be found. The number of data bases relevant to such information are numerous and much overlap occurs; the researcher must learn which ones are pertinent to their particular needs. A table of some of the major ones considered by the IJC/WQB Toxic Substances Committee and noted in the Koba Association Report are presented in Table 2. The list here is chosen on the basis that each offers information on all of physical-chemical properties, toxicology and environmental effects.

Table 2: Data Bases with Characteristic Information

BIOSIS	Biological Abstracts and Bioresearch Index
CDA	Comprehensive Dissertation Abstracts
CHEM ABS	Chemical Abstracts
CTCP	Clinical Toxicology of Commercial Products
CURR/CONT	Current Contents
ECDIN	Environmental Chemicals Data and Information Network
ENVIROLINE	
HEEDA	Health Effects and Environmental Data Analysis System
IRPTC	International Register of Potentially Toxic Chemicals
IRLG	Interagency Regulatory Liaison Group Skills Inventory
ISHOW	Information System for Hazardous Organics in Water
KIRK-OTHMER	Encyclopedia of Chemical Technology
MCMR	Michigan Critical Materials Register (CESARS)
NSF-HCL	National Science Foundation-Hazardous Chemical List
NTIS	National Technical Information Service
NURP	National Urban Runnoff Program
OA	Oceanic Abstracts
OHMTADS	Oil and Hazardous Materials Technical Assistance Data System

OCPDB	Organic Chemical Producers Data Base
POLLUTION	Pesticide Document Management System
PDMS	Diffraction Search Match
SAFETY	Safety Science Abstract Journal
SCISSEARCH	Science Citation Index
SSIE	Smithsonian Science Iformation Exchange
TITVS	Textile Abstracts
TULSA	Petroleum Abstracts
WORLDTEX	World Textiles

In addition, there are some bases which do not indicate all of the physical-chemical properties, toxicology and environmental effects but which are more comprehensive than many of the above. A few of these are:

MEDLINE	Medical Literature On-Line
NIOSHTIC	National Institute of Occupational Safety and Health: Technical Information Centre
RTECS	Registry of Toxic Effects of Chemical Substances
TOXLINE	Toxicology Information On-Line
WDROP	Water Distribution Register of Organic Pollutants

Two particular data bases from the above deserve additional comment. IRPTC is a product of the United Nations Environmental Program and is intended, ultimately, to provide international computer access to assessment related data. Development and review of the input data will be by national correspondents; the Canadian representative is the Department of National Health and Welfare (NH&W). At present, the data bases is not automated but plans exist to make it so. Also under consideration in NH&W is the possibility of placing selected data from this data base on their own MINISIS data base.

Another system (as opposed to an individual data base) is the U.S. - EPA/NIH Chemical Information System (CIS). this is a collection of data bases plus a Structure and Nomenclature Search System (SANSS) which can search the other bases if only to identify which compounds are present there. Another, projected subsystem, CSIN, of CIS, is intended to provide for a broader output (i.e. the data itself) from the approximately eighty data bases involved through interaction with the one search system.

It should also be pointed out, to Canadian users, that CISTI can search many of these data bases in addition to Chemical Abstracts and Biological Abstracts, for a nominal fee. There are also commercial firms which offer a similar service.

The foregoing has been intended to stimulate discussion on data bases relevant to hazard assessment in the Canadian situation. Consideration should be given by those in appropriate fields, including aquatic toxicology, about whether a national data base should be created and if so, what that data base should contain. The Department of the Environment is considering such a system and would like input in the form of responses to questions such as presented below:

- is there a demand for a data base with general access or do present ones suffice?
- what purposes should such a base serve?
- should such a data base include only laboratory data or should it be expanded to include environmental levels of effects and exposure?
- under what headings should the data be organized and by what parameters should data be searchable?
- what sort of data quality assurance should be built in? Should it be provided by the data generator or should all data be included and noted as "unconfirmed" etc.?

Any response to these questions, or other relevant comments can be forwarded to:

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L7R 4A6
Tel. (416) 637-4222

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Beak Consultants Limited, Vancouver, B.C.	S. Spohn	Rainbow trout	Acute toxicity	Industrial, pulp and paper, paperboard and mining effluents Flocculating agents
Beak Consultants Limited, Toronto, Ont.	R. Scroggins D. Littleford	Rainbow trout *Daphnia magna* *Chlorella vulgaris* *Selenastrum capricornutum*	Acute toxicity Reproduction failure Growth inhibition	Chlorate mud leachates Kraft bleachery effluents Deforming agents Surfactant agents Industrial effluents Sulphite mill effluents
Beak Consultants Limited, Mobile Laboratory #1	R. Scroggins	Rainbow trout Smallmouth bass Yellow perch American flagfish *Chlorella vulgaris* *Gammarus fasciatus* *Daphnia magna*	Acute toxicity of fish and invertebrates Embryo development and egg hatchability Algal growth inhibition Invertebrate growth inhibition Invertebrate reproductive stress Algal photosynthetic inhibition Fish avoidance behavior Fish taintability Fish bioconcentration	Steel plant effluent
Beak Consultants Limited, Mobile Laboratory #2	R. Scroggins D. Littleford	Rainbow trout	Acute toxicity	Kraft mill internal and external process streams Effectiveness of pilot secondary treatment plant

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Bio-Environmental Services Ltd.	R.S. Howarth P.H. Heinermann	*Salmo gairdneri* *Salmo salar* *Salvelinus fontinalis* *Jordanella floridae* *Daphnia magna*	Acute and chronic toxicity, life cycle studies, avoidance, test protocol development	Mine tailings effluent, cationic and anionic polyelectrolytes
	R.S. Howarth A.J. Burt J.A. Joyner	*Catastomas commersoni* *Sphaerium favale* *Pisidium* sp. *Helisoma* sp. *Daphnia pulex*	Bioaccumulation/bioconcentration studies	Aqueous and sediment leachate solutions of 2,4-D DMA
	D.M. Casson A.J. Burt R.S. Howarth	*Salmo gairdneri*	Fish flesh tainting, organoleptic and threshold odour magnitude estimations	Pulp and paper mill, and organic chemical manufacturing, process and waste waters
	A.J. Burt J.A. Joyner	*Salmo gairdneri* *Notemigonus crysoleucas* *Myriophylum spicatum*	Acute toxicity	2,4-D DMA Simazine Dichlobenil
	A.J. Burt D.M. Casson R.S. Howarth	*Salmo gairdneri*	Acute toxicity	Effluents of mine, oil refinery, pulp and paper mill, food processing, agriculture, chemical and manufacturing industries; ozonated oil refinery effluents

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Canada Centre for Inland Waters, Burlington, Ontario	K.K. Kwan B.J. Dutka	*Spirillum volutans* Photobacterium phosphoreum (Microtox) *Pseudomanas aeruginosa* *Aeromonas hydrophila* Mixed isolates from domestic and industrial effluents	pH effects Density effects Temperature effects Reproducibility Single chemical effect Combined chemical effect LC$_{50}$, EC$_{50}$	Hg^{++} Zn^{++} Cn^{++} Ni^{++} Pb^{++} Phenol 2,4-D 3,5 Dichlorophenol Ceyttriethylammonium chloride Cn Cd Mn Sn
Eco-Recherches Inc. (C-I-L), Pointe Claire, Québec	Ecotoxicologie R. Van Coillie C. Thellen R. Schoenert P. Benoit R. Roy N. St-Louis L. McLaughlin Y. Roy	Poissons: *Salmo salar* *Salmo gairdneri* *Salvelinus fontinalis*	Toxicite létale Histopathologie Bio-accumulation Toxicité sout-létale (détection, comportement, capacité natatoire, respirométrie, récupération)	Effluents industriels Produits chimiques Produits chimiques Effluents divers + pluies acides
		Oeufs de poisson (salmonidés)	Toxicité sous-létale et retardée	Produits chimiques et pluies acides
		Invetébrés (daphnies, gammaries, etc.)	Toxicité sout-létale Bio-accumulation	Effluents divers Produits chimiques
		Algues	Croissance, assimilation ^{14}C, fluorescence Toxicité retardée	Effluents industriels Produits chimiques
		Ecosystème expérimental	Bio-accumulation Transfert trophique Effets additifs	Produits chimiques

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Department of Fisheries and Oceans, Pacific & Freshwater Fisheries, Great Lakes Biolimnology Laboratory, Canada Centre for Inland Waters, Burlington, Ontario	P.T.S. Wong Y.K. Chau	Algae, phytoplankton	Structure-toxicity relationship	Tin, triaryl phosphates
	O. Kramar	Bacteria, fungi	Methylation	Lead, tin
	J. Maguire	Fish, algae	Occurrence	Methylated tin and lead compounds
		Microorganisms	Metabolism, toxicity, pH	Lead, selenium, arsenic and mercury
	A. Niimi B. Oliver	Fish	Structure-kinetics relationships	Chlorobenzenes, PCB's
	L. Luxon	"	Blood chemistry	Methods development
	L. Lowe-Jinde	"	Environmental levels	Mercury
	S. Campbell	"	Whole body-eggs	Major contaminants
	U. Borgmann	Copepods	Metal speciation	Cn, Cd, Pb
	K. Ralph	Rotifers Daphnia	Growth Efficiency	
	P.V. Hodson B.R. Blunt J. Hilton (Guelph) G. Dixon (Waterloo) K.L.E. Kaiser	Fish	Structure-toxicity relationships Toxicity and metabolism Biochemical techniques for fish health assessment	Para-phenols, chloro-phenols, chloro benzenes Selenium (dietary and water-borne)
	S. Millard	Model ecosystems Planktonic	Contaminant flux, adsorption, desorption, accumulation, sedimentation, volatilization	PCB - 1242 1254 HCB
	M. Munawar R.L. Thomas A. Murdoch	Natural phytoplankton	Sediment associated contaminants	Heavy metals

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT	
Environment Canada Environmental Protection Service, Aquatic Toxicology Laboratory, 14317-128 Avenue, Edmonton, Alberta T5L 3H3 403-420-2610	Art Beckett Walt Golebiowski Penny Fowler Sharon Egglestone Cathy Watson	*Salmo gairdneri* *Daphnia pulex* *Selenastrum capricornutum* *Gasterosteus aculeatus* *Artemia salinas* *Bracydanio rerio*	Acute and sublethal effects Biostimulation and inhibition Hazard assessment screening (under development) Multigeneration effects Marine toxicity testing Toxicity of effluent fractions	Industrial effluents Municipal effluents Drilling discharges Toxicity testing of commercial materials and chemical compounds	
Environment Canada Environmental Protection Service, Pacific Region	Aquatic Toxicity Laboratory	Rainbow trout underyearlings	Routine acute lethal static, 96 hr LC_{50}, LT_{50}	Pulp and paper whole mill effluent Leachate-municipal refuse: - hogfuel - mining Municipal sewage Municipal stormwater	Regularly
			Regulatory (Legal) acute lethal static, 96 hr LT_{50}	Any of the above	
			Routine acute lethal flow through, 96 hr LC_{50}	Municipal sewage Municipal stormwater	Occasionally
		3 spined stickleback	Routine acute lethal static, 96 hr LC_{50}	Marine discharge: - pulp and paper discharges (eg. W.B.L.)	

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Environment Canada Environmental Protection Service, Quebec Region	Toxicity group	*Salmo gairdneri* *Selenastrum capricornutum* Microtox	LC_{50}, LT_{50}, EC_{50} Bioaccumulation	Industrial effluents Leachate studies Water impact studies
Environment Quebec Biology Division, Ste-Foy, Quebec	G. Joubert R. Cardin L. Tremblay	Algae: *Selenastrum capricornutum*	IC_{50}-Chronic sublethal toxicity	Industrial effluents Solid residues (lixiviation) Leachates Chemicals Natural waters
		Daphnia magna	IC_{50}-Acute toxicity	Industrial effluents Solid residues (lixiviation) Leachates Chemicals
		Daphnia pulex	IC_{50}-Acute toxicity	Comparison with *D. magna*
		Microtox®	EC_{50}-Acute toxicity	Industrial effluents Solid residues (lixiviation) Leachates Chemicals
	G. Joubert L. Tousignant	Zooplanctonic Crustaceans	Structure of communities and distribution in lakes	Acid rains

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E.V.S. Consultants Limited, North Vancouver, B.C.	G.A. Vigers	Amphipods, worms, threespine stickleback	Mortality	Contaminated marine sediments containing priority pollutants
		Herring, chum and coho salmon	Acute lethality, preference/avoidance salinity, D.O., loading density	Sulphite mill effluent
		Herring larvae	Morphology, growth and development	Crude oil, and oil dispersants, bleached kraft mill effluent
		Herring juveniles	Growth mortality	Bleached kraft mill effluent, P.C.B.'s, P.C.P.'s
		Shrimp: (*Crangon communis*)	Parasitism, sex - mortality	Dredge spoil contaminated wood waste, P.C.P.'s
		Threespine stickleback	Mortality	Oil dispersants, S.E.T.'s drilling muds, dredge spoils, P.C.P. wood waste effluent
		Prickly sculpin	Bioaccumulation, mortality	Chlorophenols
		Rainbow trout	Mortality	Chlorinated organics, heavy metals, mining, pulp mill, petroleum, refinery, municipal, metal plating effluents, oil dispersants S.E.T.'s

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E.V.S. Consultants Limited, North Vancouver, B.C.	P.M. Chapman	Several species of marine and freshwater oligochaete worms	Acute toxicity (96 h LC_{50}) sublethal respiratory effects Comparative tolerances Varying temp., pH, salinity	NaPCP, Hg, Cd, sewage, black liquor from pulp mills
	D. Konasewich		Literature reviews on pathways, processes, transformations, fate and effects of environmental contaminants Code of good practice for PCP usage and disposal	U.S. EPA. 129 priority pollutants Chlorinated organics and isomers from pulp mill effluents PCP
	E.R. McGreer	*Macoma balthica* *Mytilus edulis*	Bioavailability Toxicity of contaminated substrates Sublethal effects	Heavy metals Dredge spoils Mine tailings PCB's
		Pacific cod *Gadus macrocephalus*	Growth, development of egg stages Toxic effects	Heavy metals Contaminated dredge spoils

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E.V.S. Consultants Limited, North Vancouver, B.C.	B.J. Reid	*Macoma balthica* *Capitella capitata*	Bioavailability Sublethal effects	Cadmium sulphide Phases in marine sediments
		Prickly sculpin	Bioavailability	Mine tailings Cd, Pb, An, Cu
		Pacific herring	Egg development, hatchery, growth Acute, sublethal toxicity	Bleached kraft pulp mill effluent
		Rainbow trout	Mortality	Industrial effluents
		Threespine stickleback	Mortality	Industrial effluents
Forest Pest Management Institute, Sault Ste. Marie, Ontario	P. Kingsbury S. Holmes D. Kreutzweiser	Rainbow trout Brook trout	Acute toxicity	Current and potential forest pest control agents and formulation components
Freshwater Institute, 501 University Crescent, Winnipeg, Man. R3T 2N6 Ecosystem Toxicology Section	M.K. Friesen	*Hexagenia* (Ephemeroptera)	Nymphs: toxicity of contaminated sediment Eggs: hatchability	Permethrin
	S.G. Lawrence M.H. Holoka	Algae-protozoan food chain maintained in chemostats	Numbers, biomass, cell morphology, bioaccumulation	Cadmium
	S. Leonhard	*Orconectes virilis* *Daphnia* sp.	Uptake of calcium in post moult stage	Acid

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AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Freshwater Institute, 501 University Crescent, Winnipeg, Man. R3T 2N6 Neuro-/Endocrine Toxicology Section	T.J. Hara S.B. Brown R.E. Evans B.E. Thompson B. Zielinski	Salmonid fishes (rainbow trout, whitefish, etc.)	Neuro-/Endocrine system: histopathology and plasma dynamics of glucose and hormones (thyroid, adrenocorticosteroid and sex steroids) Chemoreception and thereby mediated behaviour (feeding, spawning, etc)	Acid stress and heavy metals
Marine Ecology Lab, Department of Fisheries and Oceans, Beford Institute, Dartmouth, N.S. B2Y 4A2	P.G. Wells D. Mackay S. Abernethy	*Artemia salina* (larvae) Marine copepods (*Pseudocalanus*, *Acartia*, *Eurytemora* primarily) *Homarus americanus* (larvae, post-larvae) Other crustaceans (plankton, when available)	Lethality Swimming behavior Rate of development Molting success	Oil spill dispersants Oil dispersions and fractions Chemically dispersed oils Reference toxicants

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Noranda Research Centre	M.R. Speyer	Rainbow trout	Acute lethality 96 hr flow-through	CN^-, SCN, CNO
		Rainbow trout	Acute lethality 96 hr flow-through	Mn ($MnSO_4$) water hardness effects
		Rainbow trout	21-day growth studies	Mn ($MnSO_4$) water hardness effects
	C. Wood R. Prairie	*Mytilus edulis*	Bioaccumulation of heavy metals-*in situ* studies	Cd, Zn, Pb
Ontario Ministry of the Environment, Sudbury, Ont.	B. Bowman W. Keller	Rainbow trout Speckled trout Rainbow trout Laketrout	*In-situ* caging study 96 hr lethality *In-situ* caging eggs and alevins	Zinc Mine tailings NH_3/TDS Al and H^+ in acidic lakes

SURVEY OF AQUATIC TOXICITY RESEARCH

AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Proctor and Gamble Co., Environmental Safety Dept., c/o Michael Lewis A110B ITC, Cincinnati Ohio 45217	Toxicity research laboratory	Freshwater and marine algae	Algistatic concentration Algicidal concentration	Various surfactants Detergent builders Metals/mixtures of above
		Midge larvae planaria	LC_{50}'s on midge, hatchability, pupation, larval length, adult emergence, egg production	Various surfactants Detergent builders Metals/mixtures of above
		Daphnia magna *Gammarus* sp.	LC_{50}'s, reproduction, growth etc., sediment tox tests	Various surfactants Detergent builders Metals/mixtures of above
		Fish - various species	Sister chromatid Exchange Acute tox tests Chronic tox tests Bioconcentration Sediment tox tests	Various surfactants Detergent builders Metals/mixtures of above
Pulp and Paper Research Institute of Canada, Pointe Claire, Quebec	T. Kovacs	Rainbow trout	Acute toxicity Bioconcentration Fish tainting Larval Development	Pulp mill effluents Chlorinated organics Phenolics

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AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
Brock University Biological Sciences Dept., St. Catharines, Ontario	Mike Dickman	Acidophilic diatoms	pH	Acid rain
Contract Research with CCIW	Dickman and Ciolfi	Phytoplankton community	Impact of 2,4-D on pond phytoplankton community	2,4-D
University of Calgary, Calgary, Alberta	H. Hobe B.R. McMahon	*Catostomus commersoni*	Acid-base and ion regulatory physiology, cardiovascular and respiratory physiology	H^+ (pH 4-4.5)
University of Guelph, Dept. of Zoology	J.B. Sprague and graduate students R.W. Bradley N.J. Hutchinson C.R. Macdonald and Research Associate, W.P. Banas	Rainbow trout American flagfish	Metal toxicity interacting with water hardness, alkalinity and pH Lethal and sublethal Acclimation to toxicants Cadmium in Artic systems	Metals Arsenic Cyanide Parathion
Dept. of Environmental Biology	K. (Smith) Day	Zooplankton	Ecosystem study	Pesticides: atrazine methoxychlor permethrin
Dept. of Nutrition	J.W. Hilton	Rainbow trout	Chronic growth studies Nutrient relationship to toxicity response	Heavy metals Pesticides in the diet

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AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
University of Illinois, Newmark Civil Engineering Lab., 208 N. Romine, Urbana, Illinois 61801 (work conducted at University of Calgary, Alberta, and Sheep River, Alberta)	L.L. Osborne	Rainbow trout Epilithic Communities Bacteria Macroinverts	LC_{50} - distribution 1^o Prod.-respiration Abundance Structure-composition Abundance-chlorophylla	HOCl HOCl 2^o Chlorinated sewage 2^o Chlorinated sewage 2^o Chlorinated sewage
Lakehead University, Thunder Bay, Ontario	Biology Department members	American flagfish Rainbow trout	Bioaccumulation Bioconcentration Depuration Acute toxicity Sublethal toxicity	Chlorinated organics (benzenes, phenols)

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AGENCY OR LOCATION	INDIVIDUAL OR GROUP	TEST ORGANISM	PARAMETERS INVESTIGATED	TOXICANT
McMaster University, Dept. of Biology, Hamilton, Ontario	G. McDonald D. Lauren	Rainbow trout	Acute and chronic toxicity	Alkaline and acid environments at high and low Ca^{++} Heavy metal (i.e., copper)
	C.M. Wood	*Salmo gairdneri* *Catostomus commersoni* Crayfish	Acute toxicity Toxic interactions	Acid stress (H^{+}) Zinc Zinc and acid (H^{+}) interactions
	J.S. Goudey	Unicellular green algae *Chlorella* sp. *Chlamydomonas* sp. *Selenastrum quadricursla* *Oocystis coelustrum*	Acute and chronic toxicity of metal mixtures Kinetics of metal absorption/assimilation pH stress Nutrient stress Temperature stress	H^{+}, Cu, Cd, Ni, Co, Sn
University of New Brunswick, North American Salmon Research Centre, St. Andrews, New Brunswick	C.B. Schom	*Salmo salar*	Acute toxicity chronic developmental defects	Low pH Cadmium Pesticide (Azodrin)

SURVEY OF AQUATIC TOXICITY RESEARCH

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University of Ottawa	R.P. Moody	Unicellular, green algae (eg., *Chlamydomonas reinhardii*)	Population growth Cell motility ATP synthesis Cell ultrastructure Photosynthesis ($^{14}CO_2$ fixation, DCIP reduction, fluorescence induction) Algal accumulation and degradation of pesticide residues	Pesticides (eg., fenitrothion, matacil, 2,4-D) Pesticide formulation cosolvents (eg., Aerotex 3470, Atlox 3409F, Dowanol, Cyclitol, Diluent-585, Nonyl Phenol) Petroleum hydrocarbons (eg., benzene, naphthalene and methyl-benzenes (toluene, xylene) and methyl-naphthalenes)

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Université du Québec, INRS-Eau, Sainte-Foy	S.S. Bates	*Chlamydomonas variabilis* *Scenedesmus subspicatus* (Chlorophyceae)	Mechanisms of trace metal adsorption and assimilation Metal speciation and toxicity Complexation by fulvic acids pH effects	Zn, Cu
	P. Couture	*Chlamydomonas variabilis* *Selenastrum capricornutum* (Chlorophycée)	A-Changements dans la toxicité originale des effluents -effet du traitement (stérilisation, filtration) -effet de la conservation B-Recherche de méthodes rapides d'évalution de la toxicité -ATP -Fluorescence -C^{14}	Effluents $CuSO_4$ NaN_3
University of Quebec, Montreal, P.Q.	Members of Chemistry Department	Rainbow trout cells	Protein and nucleic acid (DNA and RNA) synthesis Incorporation of radio-labelled precursors	Pb, Cd, PCB, etc.

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University of Toronto, Institute of Environmental Studies, Main Campus, Toronto, Ont.	P.M. Stokes Mr. Turner	*Mangeotas* sp. *Spirogyra* sp.	^{14}C studies Long term growth effects	H^+ Al
Department of Zoology, Main Campus	H.H. Harvey	Native Ontario fishes	Acute toxicity Bioaccumulation Natural environment Bioconcentration	H^+ Al Mn
Life Sciences Division, Scarborough Campus	K. Lee	Freshwater and marine phytoplankton and bacteria	Primary production Nutrient uptake kinetics Heterotrophic responses	Vanadium
University of Victoria, Dept. of Biochemistry & Microbiology	A.T. Matheson J.A. McCarter R.W. Olafson J.T. Buckley W.W. Kaye E. Ishiguro T.J. Trust	Coho salmon Rainbow trout	Toxicity of copper, cadmium and zinc to salmonids Metallothinonein levels in relation to tolerance and exposure, kinetics of metallothinonein induction in fish Disease resistance during metal exposure	Copper, zinc, cadmium

SURVEY OF AQUATIC TOXICITY RESEARCH

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York University, Dept. of Biology, Downsview, Ontario	B.C. Birmingham B. Colman	Macrophyte: *Myrio phyllum spicatum* Weed-infested artificial ponds, *Myriophyllum spicatum* Phytoplankton Zooplankton	Growth rate Photosynthesis Respiration Nitrogen fixation Herbicide uptake and metabolism Fate and persistence of herbicide in water, mud and plants in an environment simulation	2,4-D-Butoxyethanol 'ester (Aquakleen®)

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